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Monitoring treatment response in Crohn's disease

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications:

- I af Björkesten CG, Nieminen U, Turunen U, Arkkila PE, Sipponen T, Färkkilä MA. Endoscopic monitoring of infliximab therapy in Crohn's disease. *Inflammatory Bowel Diseases* 2011;17(4):947-53.
- II af Björkesten CG, Nieminen U, Sipponen T, Turunen U, Arkkila P, Färkkilä M. Mucosal healing at 3 months predicts long-term endoscopic remission in anti-TNF-treated luminal Crohn's disease. *Scandinavian Journal of Gastroenterology* 2013;48(5):543-51.
- III Molander P, af Björkesten CG, Mustonen H, Haapamäki J, Vauhkonen M, Kolho KL, Färkkilä MA, Sipponen T. Fecal calprotectin concentration predicts outcome in inflammatory bowel disease after induction therapy with TNF α blocking agents. *Inflammatory Bowel Diseases* 2012;18(11):2011-7.
- IV af Björkesten CG, Nieminen U, Turunen U, Arkkila PE, Sipponen T, Färkkilä MA. Surrogate markers and clinical indices, alone or combined, as indicators for endoscopic remission in anti-TNF-treated luminal Crohn's disease. *Scandinavian Journal of Gastroenterology* 2012;47(5):528-37.

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*I, III: Wolter Kluwer Health; II, IV: Informa Healthcare

ABBREVIATIONS

anti-TNF	anti-tumour necrosis factor- α antibodies
ASCA	anti- <i>Saccharomyces cerevisiae</i> antibodies
ATG16L1	autophagy-related protein 16-1 gene
CARD	caspase-activating recruitment domain
CBir1	anti-flagellin antibody
CD	Crohn's disease
CDAI	Crohn's disease activity index
CDEAS	Crohn's disease endomicroscopic activity score
CDEIS	Crohn's disease endoscopic index of severity
CLE	confocal laser endomicroscopy
⁵¹ Cr-EDTA	chromium-ethylene diamine tetra-acetic acid
CRP	C-reactive protein
CT	computed tomography
DBE	double-balloon enteroscopy
ELISA	enzyme-linked immunosorbent assay
ESR	erythrocyte sedimentation rate
GI	gastrointestinal
HBI	Harvey-Bradshaw index
Hct	haematocrit
hsCRP	high sensitivity C-reactive protein
kDa	kilodalton
IBD	inflammatory bowel disease
IBD-HOT	Inflammatory bowel disease – Health Outcome of Treatment study
IBD-U	inflammatory bowel disease – unclassified
IBS	irritable bowel syndrome
IFN	interferon
IL	interleukin
MAP	<i>Mycobacterium avium paratuberculosis</i>
MRI	magnetic resonance imaging
NBI	narrow-band imaging
NOD	nucleotide oligomerisation domain
ompC	outer-membrane porin C
pANCA	anti-neutrophil cytoplasmic antibody with perinuclear staining pattern
PCDAI	paediatric Crohn's disease activity index
PDAI	perianal Crohn's disease activity index
PEG	polyethylene glycol
PMN-e	polymorphonuclear neutrophil elastase
ROC curve	receiver operator characteristic curve
SBCE	small bowel capsule endoscopy
SBE	small bowel enteroclysis
SBFT	small bowel follow-through
SES-CD	simple endoscopic score for Crohn's disease
⁹⁹ Tc-DPTA	diethylene triaminepentaacetic acid
Th	T-helper
TNF α	tumour necrosis factor- α
UC	ulcerative colitis
US	ultrasound

ABSTRACT

Background

Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD) most commonly affecting the colon and terminal ileum. It has typically a relapsing and remitting course, with its worsening characterised by diarrhoea, abdominal pain, and bloody stools. One of the fundamental goals in the treatment of CD should be mucosal healing, because complete disappearance of mucosal ulcerations has been associated with a significantly better outcome: improvement of quality of life and reduction of hospitalization and need for surgery. Endoscopic indices such as the simple endoscopic score for Crohn's disease (SES-CD) have been developed and validated for assessment of luminal inflammatory activity. Traditional assessment of CD activity, however, has been based on symptoms and clinical signs, although symptoms sometimes fail to correlate with bowel inflammatory activity. The role of clinical indices such as the Crohn's disease activity index (CDAI) and the Harvey-Bradshaw index (HBI), and surrogate laboratory markers such as faecal calprotectin to indicate CD remission determined by endoscopy is unsettled. Furthermore, studies on predictive markers for endoscopic outcome in CD treated with anti-tumour necrosis factor- α antibodies (anti-TNF) are limited.

Patients and methods

Data on 71 patients with active luminal CD who were treated with infliximab underwent retrospective analysis to assess any features predicting long-term outcome (Study I). All patients underwent endoscopy at baseline and at three months from start of infliximab, whereas further twelve-month endoscopic follow-up data were available for 57 patients. CD activity was scored by reassessment of the existing endoscopy reports.

Study II included prospectively collected data from 42 patients with endoscopically active luminal CD treated with anti-TNF, followed up for one year from start of anti-TNF. All patients received anti-TNF for at least three months, after which they underwent endoscopic assessment. Endoscopic or clinical response legitimised anti-TNF continuation. All patients had data on either the SES-CD obtained approximately one year from start of anti-TNF, or data on bowel surgery during follow-up. Laboratory data were analysed, and clinical activity was assessed with the CDAI at baseline and in connection with the follow-up endoscopies.

To assess the predictive role of faecal calprotectin measured after anti-TNF induction, a retrospective study began that comprised 60 IBD patients, including 34 patients with luminal CD (Study III). These patients were divided into groups according to calprotectin levels measured after anti-TNF induction, and anti-TNF was continued in all those with an endoscopic or clinical response. Clinical CD activity was assessed by the HBI one year from start of anti-TNF.

Study IV included 64 CD patients with prospective follow-up data on 210 endoscopies and at least one concurrent non-invasive disease activity marker. Mucosal inflammatory activity was scored according to the SES-CD and compared with available concurrent

clinical indices and laboratory markers, and different scores were based on their combinations.

Results

Among patients continuing anti-TNF as maintenance therapy, twelve-month endoscopic remission was significantly more common in those patients who had been in endoscopic remission at three months, than in those with endoscopically active disease at three months (90% vs. 33% in Study I, $p=0.0001$; 70% vs. 17% in Study II, $p=0.003$), and the three-month SES-CD predicted twelve-month endoscopic remission with 88% sensitivity and 64% specificity (Study II). No patient with endoscopically inactive disease at three months underwent surgery during the follow-up. The calprotectin cut-off, 139 $\mu\text{g/g}$, had a sensitivity of 72% and specificity of 80% to predict one-year clinical relapse. A calprotectin decline of more than 88% during anti-TNF induction predicted clinical remission at one year with a sensitivity of 87% and specificity of 65% (Study III). Neither C-reactive protein (CRP) nor the CDAI at three months was capable of showing any connection with twelve-month endoscopic remission (Study II).

The SES-CD demonstrated a stronger correlation with calprotectin ($r=0.56$, $p<0.001$) and CRP ($r=0.56$, $p<0.001$) than with the CDAI ($r=0.40$, $p<0.001$) or HBI ($r=0.32$, $p<0.001$) (Study IV). With the use of widely accepted cut-offs, the CDAI and the HBI indicated clinical remission almost three times as often, and CRP was normal nearly twice as often as when the SES-CD indicated remission. CRP and clinical index cut-off optimisation improved only slightly their power to detect endoscopic remission. However, although faecal calprotectin alone identified endoscopic remission with 84% sensitivity and 74% specificity, it was beaten, but not statistically significantly, by a combined index based on calprotectin and the HBI (sensitivity 86%, specificity 82%) (Study IV). Although the clinical indices and CRP, used alone or in combination, proved inferior to calprotectin alone, a score based on CRP and the HBI seemed to perform better in identifying endoscopic remission than did either test separately.

Conclusions

In anti-TNF-treated active luminal CD, endoscopic remission at three months is a useful predictor for maintenance of a long-term endoscopic response. CRP and clinical indices commonly used in the assessment of disease activity and treatment response are, both alone and combined, inferior to faecal calprotectin at determining mucosal inflammatory activity and detecting endoscopic remission. In patients on scheduled anti-TNF therapy, a normal faecal calprotectin after anti-TNF induction is a predictor of sustained clinical remission. Evidently it also may be able to predict long-term endoscopic remission. Moreover, a score based on a combination of calprotectin and the HBI may function as a new tool for identifying endoscopic remission. For optimisation of anti-TNF therapy in active luminal CD in clinical practice, these study results suggest an objective inflammatory activity assessment such as ileocolonoscopy or determination of faecal calprotectin, performed as early as three months after initiation of therapy.

INTRODUCTION

Crohn's disease (CD) is a disabling transmural and segmental chronic inflammatory bowel disease (IBD) with a relapsing and remitting course. Its inflammatory lesions can affect the entire gastrointestinal (GI) tract, in contrast to the other major type of IBD, ulcerative colitis (UC), which is limited solely to the colon (Baumgart and Sandborn 2007). The aetiology and pathogenesis of CD is incompletely known, but the most widely accepted hypothesis is that it arises from interactions between immunoregulatory, genetic, and environmental factors (Baumgart and Carding 2007). Exacerbations in CD are characterised by symptoms such as diarrhoea, abdominal pain, and rectal bleeding. Assessment of CD activity has traditionally been based on symptoms and clinical signs, although symptoms sometimes fail to correlate with bowel inflammatory activity. Despite great advances in development of medical therapy during the last two decades, CD is still considered incurable, and in many patients leads to multiple complications (Loftus 2006). Although surgery as a treatment option is limited to complications such as strictures and fistulae, despite optimized medical therapy, surgery will ultimately be necessary in up to 70% of cases (Bernell *et al.* 2000).

During the era of therapy with anti-tumour necrosis factor- α antibodies (anti-TNF), complete disappearance of mucosal ulcerations has been associated with favourable outcome, and after initiation with anti-TNF, complete mucosal healing has been the only factor predicting long-term steroid-free remission (Hommes and van Deventer 2004, Rutgeerts *et al.* 2007). Accordingly, for assessing CD activity, for tailoring therapy, and for measuring treatment response, objective determination of inflammatory activity should be essential. In anti-TNF-treated CD, the cost-effect dimension and the fact that a significant proportion of patients may fail to respond to therapy make identifying predictors of response to anti-TNF also important. These predictors, by allowing a better selection of patients, would thereby reduce the associated health care costs. The gold standard for assessment of luminal inflammation in CD is endoscopy with biopsies, but the role of endoscopy as a disease-activity monitoring and prognostic tool in anti-TNF-treated CD is insufficiently established.

Additionally, because endoscopic procedures are time-consuming, expensive, and unpleasant for patients, surrogate markers of mucosal inflammation are currently under intensive investigation. To reveal intestinal inflammation, conventional laboratory markers in the blood such as haemoglobin, C-reactive protein (CRP), or erythrocyte sedimentation rate (ESR) have proved insufficiently sensitive (Cellier *et al.* 1994, Desai *et al.* 2007). Faecal calprotectin, an inflammatory product of the intestinal mucosa, has repeatedly been correlated in luminal CD with both endoscopic and histological findings; low calprotectin concentration has also served as a surrogate marker for endoscopically and histologically inactive disease (Roseth *et al.* 1999, Sipponen *et al.* 2008a). The role of

surrogate markers of inflammatory activity is, however, still an open question; for example their cut-off values for remission in anti-TNF-treated luminally active CD are unclear (Lewis 2011). The existing non-invasive scores developed for disease activity assessment are infrequently used in clinical practice. These scores are mainly based on clinical symptoms and show only weak correlations with endoscopically determined inflammatory activity (Cellier *et al.* 1994, Sipponen *et al.* 2008c). Few have attempted to develop combined scores consisting of clinical findings and symptoms and laboratory markers to improve the identification of luminal inflammatory activity (Langhorst *et al.* 2008).

This thesis aims to evaluate the role of endoscopy in monitoring maintenance anti-TNF therapy and predicting long-term response to anti-TNF, to analyze the role of surrogate markers and clinical indices in comparison to endoscopic disease activity in active luminal CD, and further to develop methods for predicting long-term efficacy of anti-TNF treatment. Additionally, it aims to assess the accuracy of surrogate and clinical indices, alone or in combination, in identifying endoscopically determined remission.

REVIEW OF THE LITERATURE

Crohn's disease, also known as regional enteritis, terminal ileitis, granulomatous ileitis, hyperplastic ileitis, and chronic ulcerative ileitis, is named after the American gastroenterologist Burrill B. Crohn, who, together with his colleagues Leon Ginzburg and Gordon D. Oppenheimer, described 14 patient cases with regional enteritis over 80 years ago (Crohn *et al.* 1932). A transmural inflammatory disease of the GI mucosa capable of affecting any part of the GI tract, CD is characterized by a chronic course with phases of remission interrupted by unpredictable worsening episodes or relapses. Some patients may have chronically active disease, meaning continuously active inflammation (Baumgart and Sandborn 2007).

1 Epidemiology and outcome of Crohn's disease

CD shows a small female preponderance, gender ratios depending, however, on age and geographic region (Brant and Nguyen 2008). Typically, CD manifests in adolescents, but approximately 20% develop symptoms in childhood (Heyman *et al.* 2005, Nikolaus and Schreiber 2007). The highest incidence and prevalence rates occur in developed countries with annual incidences of up to 16.3/100,000 and prevalences of 213/100,000 (Loftus 2004, Lapidus 2006, Baumgart and Carding 2007, Bernstein and Shanahan 2008). Annually, Europe has an estimated 23,000 to 41,000 new CD cases, with the incidence still rising (Loftus 2004). Distinct north-south and west-east gradients exist within Europe, with the highest rates in northern and western countries, but the incidences in southern and eastern Europe are increasing faster (Shivananda *et al.* 1996, Burisch *et al.* 2013). The IBD incidence in Finland is globally among the highest, with recent studies reporting locally increasing but nationally more stable CD incidences during the previous decade (Manninen *et al.* 2010, Jussila *et al.* 2012). Age below 40 years, perianal involvement, and need for corticosteroid therapy at the time of diagnosis are factors predicting a more disabling course (Beaugerie *et al.* 2006). The life expectancy of patients with CD is slightly lower than average (Persson *et al.* 1996, Wolters *et al.* 2006, Manninen *et al.* 2012). Despite improved medical knowledge and available therapy, no significant decrease has occurred in mortality for CD patients over the last several decades (Loftus 2006).

2 Aetiology and pathogenesis of Crohn's disease

Despite marked improvement in the understanding of immunological mechanisms during recent years, the exact aetiological factors involved in the pathogenesis of CD remain elusive. The prevailing hypothesis is that a disturbed interaction of the host

immune system with its commensal microbiota and other luminal agents leads to damaged bowel mucosa (Baumgart and Carding 2007).

2.1 Genetics

The strongest risk factor for CD is having a relative with that same disease. First-degree relatives of patients with CD have a 12- to 15-fold greater risk for developing the disease than do people of comparable age in the general population (Simmons *et al.* 2000). Patients with CD have a first-degree relative with IBD in up to 22% of cases (Gaya *et al.* 2006). The inherited predisposition is also demonstrated by the higher prevalence of CD among Jewish people than among any other ethnic group, and by a pooled concordance of 36% in monozygotic twins (Rosenstiel *et al.* 2009). Several genes have been related to CD. Those genes are related to innate pattern-recognition receptors, to epithelial barrier homeostasis and maintenance of epithelial barrier integrity, to autophagy, and to lymphocyte differentiation. Thus far, the strongest and most often replicated associations with CD have been done with CARD15/NOD2, IL23R, and ATG16L1 genes. The gene encoding caspase-activating recruitment domain 15 (CARD15) – also known as the nucleotide oligomerisation domain 2 (NOD2) – plays a key role in innate host defence, and its mutations associate strongly with CD affecting the ileum and particularly with stricturing disease (Gaya *et al.* 2006). CARD15 seems to be not only a susceptibility gene, but also a disease-modifier gene for CD. Furthermore, studies have revealed that CD susceptibility is associated with several polymorphisms of the IL-23 receptor (IL-23R) gene locus and single nucleotide polymorphisms in the regions of the autophagy gene ATG16L1 (Duerr *et al.* 2006, Cadwell *et al.* 2008). Recently, a genome-wide association meta-analysis defined more than 70 distinct CD susceptibility loci (Franke *et al.* 2010).

2.2 Environmental factors

Diet, microbiota, use of nonsteroidal anti-inflammatory drugs, and high hygiene level have all attracted study interest as triggers for CD. For example, diets high in sucrose, refined carbohydrates, and omega (ω)-6 polyunsaturated fatty acids, and diets low in fruits and vegetables seem associated with increased risk for CD (Neuman and Nanau 2012). Nevertheless, the associations between cigarette smoking and CD are by far the best studied: current smoking elevates the risk for developing the disease, it adversely affects the course of the disease, raises exacerbation rates, promotes complications and risk for surgery; and smoking cessation may lead to lessening of disease severity (Cosnes *et al.* 2001, Mahid *et al.* 2006, Higuchi *et al.* 2012).

2.3 Role of microbiota

Metagenomic research suggests that up to four major bacterial phyla (Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria), consisting of thousands of mostly anaerobic species, colonise the human gut. Variation in bacterial-species diversity in the gut depends upon temporal, individual, dietary, and drug-induced factors (Costello *et al.* 2009, Turnbaugh *et al.* 2010, Muegge *et al.* 2011, Dethlefsen and Relman 2011). However, healthy intestinal microbiota variation is generally stratified and not continuous (Arumugam *et al.* 2011). In patients with CD, studies have showed clustering and reduced diversity, especially within the Firmicutes and Bacteroides phyla; reduction in the Firmicute *Faecalibacterium prausnitzii* has been associated with increased risk for postoperative recurrence of ileal CD (Frank *et al.* 2007, Sokol *et al.* 2008, Qin *et al.* 2010, Willing *et al.* 2010). Higher levels of *Mycobacterium avium paratuberculosis* (MAP) may occur in the tissues and blood of CD patients than in controls, but despite considerable research, the role of MAP in CD pathogenesis remains inconclusive (Chiodini *et al.* 1984, Bull *et al.* 2003). MAP in CD currently attracts, however, mostly academic interest because there exists no clinically useful test to identify its presence nor any evidence to support the use of antimicrobials to eradicate it (Bernstein *et al.* 2004, Selby *et al.* 2007).

2.4 Role of immune response

In CD, the chronic autoimmune intestinal inflammatory process results from pathological interaction of the immune system with commensal enteric bacteria (Xavier and Podolsky 2007). The mucosal host defence deteriorates due to abnormalities in the innate immune response and adaptive immune system. The innate immune system provides the nonspecific defence against pathogens by means of macrophages, dendritic cells, natural killer cells, neutrophils, and the complement system. Responses of these components are inborn and not tailored to any particular immunological challenge. As a consequence of mucus biofilm insufficiency and decreased excretion of antimicrobial agents in epithelial cells, the normally tight seals between cells become leaky, resulting in increased permeability and an access of luminal antigens into the lamina propria (Buisine *et al.* 1999, Söderholm *et al.* 2002). Dendritic cells express a wide range of pattern-recognition receptors and interpret microbial patterns to direct other immune cells towards immunity or tolerance (Niess *et al.* 2005). When dendritic cells lose their ability to induce regulatory T cells, this leads to loss of tolerance of microbial antigens or to the induction of cross-reactive autoimmune responses (Sartor 2008, Iliev *et al.* 2009).

The adaptive immune system is slower than the innate immune system, and the secondary response is more specifically tailored through function of T- and B-lymphocytes. In CD, this system is thought to mediate and maintain, but probably not initiate, intestinal inflammation (Baumgart and Carding 2007). The patchy transmural inflammation characteristic of CD is associated with activation of types 1 and 17 T-helper (Th) cells in response to production of interleukins (IL) and transforming growth

factor β by antigen-presenting cells and macrophages. Th1 and Th17 cells, in turn, cause increased secretion of the pro-inflammatory cytokines IL-2, IL-17, interferon (IFN)- γ , and tumour necrosis factor- α (TNF α). These cytokines feed into a self-sufficient cycle whereby they stimulate antigen-presenting cells, macrophages, fibroblasts, and endothelial cells to produce TNF α , IL-1, IL-6, IL-8, IL-12, and IL-18 (Collison *et al.* 2010, Engel and Neurath 2010, Franke *et al.* 2010).

3 Disease classification by phenotype

Typical presentations of CD include discontinuous involvement of various portions of the GI tract and development of disease complications such as strictures, fistulae, or abscesses. At diagnosis, about half the patients present with purely terminal ileitis, in approximately one-quarter both the terminal ileum and colon are affected, and in about one-quarter only the colon is involved (Baumgart and Sandborn 2007). In less than one-tenth of all patients, CD may affect the ileum out of reach of ileocolonoscopy or involve the more proximal small bowel or the upper GI tract. Additionally, at the time of diagnosis, 15% of patients have penetrating lesions, meaning fistulae or abscesses (Van Assche *et al.* 2010a). Disease classification allows clinicians to differentiate among the features and behaviours of CD. The 2005 Montreal revision of the Vienna classification is regarded as the international standard of CD phenotype subtyping (Gasche *et al.* 2000, Satsangi *et al.* 2006) (Table 1).

Table 1. Vienna and Montreal classifications for Crohn’s disease

Age at diagnosis	A1 <40 years A2 ≥40 years	A1 ≤16 years A2 17–40 years A3 >40 years
Localisation	L1 ileal L2 colonic L3 ileocolonic L4 upper	L1 ileal L2 colonic L3 ileocolonic L4 isolated upper disease*
Behaviour	B1 non-stricturing, non-penetrating B2 stricturing B3 penetrating	B1 non-stricturing, non-penetrating B2 stricturing B3 penetrating p perianal disease modifier**

* L4 is a modifier that can be added to L1-L3 when concomitant upper gastrointestinal disease is present,
 ** The modifier “p” is added to B1–B3 when concomitant perianal disease is present.
Satsangi J, Silverberg MS, Vermeire S et al. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. Gut 2006;55:749-753. Reproduced with permission from BMJ Publishing Group Ltd.

A well-established fact is that after diagnosis of adult patients, location subtyping remains stable, whereas behaviour subtyping changes continuously, with an increasing proportion of patients progressing from inflammatory disease to stricturing or

penetrating disease (Louis *et al.* 2001, Cosnes *et al.* 2002). According to one follow-up study of new CD cases, however, changes in disease location were apparent at 5 years in 13.5% and in disease behaviour in 17.5% of patients. In ileal CD patients, stricturing complications were evident in 64%, but in only 6% of patients with colonic CD (Henriksen *et al.* 2007).

4 **Diagnosis**

As there exists no single method to diagnose CD, Lennard-Jones and Shivananda (1997) with the European IBD study group have defined macroscopic and microscopic criteria for establishing diagnosis. Macroscopic diagnostic tools include physical, endoscopic, and radiological examination, and examination of a surgical specimen. Microscopic features can only in part be analysed by mucosal biopsy, but can be completely analysed in a surgical specimen. Diagnosis is based on the finding of noncontinuous and often granulomatous intestinal inflammation. Current opinion is that diagnosis is, in practice, established by a loosely defined combination of clinical presentation, endoscopic features, radiological findings, histological appearance, surgical findings and, more recently, serological abnormalities (Van Assche *et al.* 2010a). In clinical practice, CD and UC can typically be differentiated by their clinical characteristics. The main differences between CD and UC are location and nature of the inflammatory changes (**Table 2**).

Table 2. Signs and findings differentiating Crohn’s disease and ulcerative colitis in adult patients		
	Crohn’s disease	Ulcerative colitis
Stool consistency	Often porridge-like, sometimes steatorrhoea	Often mucus-like
Tenesmus	Less common	More common
Fever	Common	Indicates severe disease
Fistulae	Common	Rare
Weight loss	Often	More seldom
Terminal ileum involvement	Common	Rare
Colon involvement	Common	Always
Rectum involvement	More seldom	Nearly always
Perianal involvement	Common	Rare
Distribution of disease	Patchy inflammation	Continuous area of inflammation
Ulcers seen in endoscopy	Deep geographic, snake-like	Continuous, superficial
Strictures	Common	Rare
Depth of inflammation	Transmural	Limited to mucosa
Granulomatous inflammation	Common	Absent
Presence of antimicrobial antibodies: ASCA, anti-Cbir1, anti-OmpC	More often	Seldom
ASCA: anti- <i>Saccharomyces cerevisiae</i> antibodies; anti-Cbir1: anti-flagellin antibody; anti-OmpC: <i>Escherichia coli</i> outer membrane porin C antibody.		
<i>Data from from Lennard-Jones JE, Shivananda S. Clinical uniformity of inflammatory bowel disease a presentation and during the first year of disease in the north and south of Europe. EC-IBD Study Group. Eur J Gastroenterol Hepatol 1997;9:353-359 and Van Assche G, Dignass A, Panes J et al. The second European evidence-based consensus on the diagnosis and management of Crohn’s disease: Definitions and diagnosis. J Crohns Colitis 2010;4:7-27.</i>		

The term “inflammatory bowel disease – unclassified” (IBD-U) is appropriate in situations where a definitive distinction between CD, UC, or other causes of colitis is impossible despite appropriate diagnostic assessment (Satsangi *et al.* 2006). Indeterminate colitis is a pathological-anatomical diagnosis reserved for pathologists to describe a colectomy specimen with overlapping features of CD and UC (Price 1978, Satsangi *et al.* 2006).

4.1 Clinical presentation

Symptoms in CD are heterogeneous and depend on disease location and behaviour. Chronic diarrhoea is the most common symptom, affecting up to 85% of patients (Sands 2004). Abdominal pain occurs in approximately 70% and weight loss in 60% of patients before diagnosis, and in CD patients with colonic disease, bloody or mucous stools or both occur in up to 50% (Lennard-Jones and Shivananda 1997). Fever, rectal pain, and fatigue may also be present. More acute presentations may occur, and acute terminal ileal CD may even be mistaken for acute appendicitis. CD can also cause unexplained anaemia, chronic non-specific symptoms resembling irritable bowel syndrome (IBS), and, in children, growth failure (Burgmann *et al.* 2006). On the other hand, as IBS is up to three times as prevalent in IBD as in the non-IBD population, symptoms compatible with IBS can sometimes dominate the clinical picture despite IBD remission (Simrén *et al.* 2002). Up to 30% of patients also present with extraintestinal manifestations such as peripheral arthropathy, axial arthritis, ocular (uveitis, episcleritis), cutaneous (erythema nodosum, pyoderma gangraenosum), or hepatobiliary disease (primary sclerosing cholangitis). Extraintestinal manifestations are most common when CD affects the colon. At diagnosis, 10% of patients have perianal fistulae (Schwartz *et al.* 2002, Van Assche *et al.* 2010a).

In physical examination, a CD patient may be underweight and even malnourished. Ileal CD can present with pain in the right lower abdomen. Palpation may indicate an abdominal mass or may cause pain. Discovery of perianal fistulae or fissures may follow anal inspection or rectal palpation. Small aphthous ulcers may be evident in the oral cavity (Van Assche *et al.* 2010a).

4.2 Laboratory tests

In the full blood count of CD patients, anaemia and thrombocytosis are the most common changes. ESR and CRP may be elevated, and albumin levels low. Stool tests for investigation of pathogenic bacteria, especially *Clostridium difficile*, and parasites are necessary to differentiate between IBD and infectious colitis. Stool tests can additionally reveal elevated levels of faecal inflammatory markers (Vermeire *et al.* 2004). Anti-*Saccharomyces cerevisiae* antibodies (ASCA) directed against *Candida albicans*, and perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) may, particularly in difficult cases, be

useful in improving diagnostic precision and differentiation between UC and CD (Standaert-Vitse *et al.* 2006). In a meta-analysis of studies involving detection of CD in 4,019 patients, positive detection of ASCA in combination with lack of pANCA resulted in 55% sensitivity and 93% specificity (Reese *et al.* 2006). Population studies have revealed a particular strength of ASCA in its predicting a complicated, severe course of the disease (Riis *et al.* 2007). Positive detection of ASCA is, however, a nonspecific finding occurring in up to 60% of patients with coeliac disease, suggesting an immune response to commensal microbes inducing mucosal damage (Ashorn *et al.* 2008). Other serum antimicrobial antibodies of IBD patients include the CD-related protein from *Pseudomonas fluorescens* (anti-I2), a flagellin-like antigen (anti-Cbir1), and *Escherichia coli* outer membrane porin C (anti-OmpC) I2 antibodies (Mow *et al.* 2004, Targan *et al.* 2005). These antibodies are detectable in about 50% of CD patients but in only 10% with UC. Anti-CBir1 expression is associated independently with small bowel, with penetrating, and with stricturing disease (Vernier *et al.* 2004).

Patients concurrently positive for ASCA, for anti-ompC, and for anti-I2 are eight times as likely as are seronegative patients to require small bowel surgery (Mow *et al.* 2004). These antibody responses may play a role in subtyping CD patients, in prediction of disease course, or in differentiation of IBD-U. Serological testing currently available may serve as a complement to diagnosis in clinical practice, but because of their inaccuracy, even the best available tests are of little use in routine clinical diagnosis (Reese *et al.* 2006). Despite huge advances in the field of CD genetics, currently no laboratory genetic test exists that can be recommended routinely for diagnosis (Van Assche *et al.* 2010a).

4.3 Endoscopy

The gold standard as a first-line diagnostic procedure for suspected CD is full ileocolonoscopy providing multiple biopsy specimens. With practice, the ileum can be reached in at least 85% of colonoscopies, which enhances diagnosis of CD in patients presenting with symptoms of IBD (Coremans *et al.* 1984). The central endoscopic features of CD are discontinuous involvement, anal lesions, and cobblestoning. Anatomical criteria of severe disease are deep ulcerations eroding the muscle layer, or mucosal detachments or ulcerations limited to the submucosa but extending to more than one-third of a specific colonic segment (right, transverse, or left colon) (Nahon *et al.* 2002).

In severe, active disease, however, full ileocolonoscopy leads to increased risk for bowel perforation, and diagnostic errors are more frequent. In such circumstances, initial flexible sigmoidoscopy is safer, and full ileocolonoscopy should be delayed until the patient's condition improves (Van Assche *et al.* 2010a). A further diagnostic limitation of endoscopy is its inability to detect disease activity beyond the mucosa.

Irrespective of the findings in ileocolonoscopy, further investigation is recommended to determine the location and extent of the disease in the small bowel and upper GI tract

(Van Assche *et al.* 2010a). CD affecting the upper GI tract is almost always accompanied by small- or large-bowel involvement. Gastric biopsies may prove useful in a patient with IBD-U, as CD may include focal active gastritis in the absence of ulceration (Witte *et al.* 1998). Because prevalence rates for CD in the upper GI tract can be high (17–75%), especially in symptomatic patients (dysphagia, chest pain, heartburn, dyspepsia, epigastric pain), some experts have suggested that all newly diagnosed patients with CD should have at least one upper endoscopy (Hommes and van Deventer 2004).

Recently, wireless small-bowel capsule endoscopy (SBCE), device-assisted enteroscopy, and new imaging modalities have offered novel possibilities for detecting inflammatory lesions in the small bowel where traditional endoscopic and radiologic approaches have limitations.

SBCE, a technique using a wireless miniature encapsulated video camera designed to examine the entire small bowel and directly visualize small bowel lesions, is useful in suspicion of small bowel CD and in assessment of its extent and severity (Fireman *et al.* 2003). In patients with IBD-U, SBCE may help distinguish between UC and CD. SBCE is superior to small bowel follow-through (SBFT), barium enteroclysis (SBE), and conventional computed tomography (CT) in establishing the diagnosis and estimating disease extent and is widely considered a first-line examination after negative ileocolonoscopy and upper endoscopy (Sandrasegaran *et al.* 2008, Tillack *et al.* 2008, Riccioni *et al.* 2012b). SBCE is, however, limited by cost and its inability to provide either tissue samples or therapy. Furthermore, because SBCE produces picture data at constant speed irrespective of the capsule's pace through the small bowel, localization of lesions is tricky. Absolute contraindications for SBCE are suspected or documented intestinal obstruction or strictures. In suspected or verified CD, potential risk for capsule retention should therefore always be considered (Hommes and van Deventer 2004).

Double-balloon enteroscopy (DBE) is a device-assisted enteroscopy technique for reaching lesions throughout the entire small bowel (Yamamoto *et al.* 2001). The scope may be inserted either orally or anally. As the availability of DBE is limited, it should be reserved for situations in which biopsy samples are vital for diagnosis or in which dilatation of strictures is required. DBE plays an additional role in retrieval of retained capsules, which may avoid surgery. Newer modalities of device-assisted enteroscopy are single-balloon enteroscopy and spiral enteroscopy (Riccioni *et al.* 2012a).

Chromoendoscopy uses various techniques during endoscopy to enhance mucosal detail and submucosal vascular pattern. It can be divided into dye-based and dye-less imaging. Although dye-based chromoendoscopy yields additional diagnostic value with a three- to four-fold higher detection rate of intraepithelial neoplasia, it is time-consuming and costly (Kiesslich *et al.* 2003, Neumann *et al.* 2011). Dye-less chromoendoscopy, also called virtual chromoendoscopy, has therefore been developed. Virtual

chromoendoscopy such as narrow-band imaging (NBI) and Fujinon intelligent colour enhancement uses light of blue and green wavelengths to enhance detail of the mucosal surface and its capillary patterns (Neumann *et al.* 2009). Confocal laser endomicroscopy (CLE) is a recently introduced endoscopic tool making it possible to carry out microscopic examination with 1000-fold magnification of the mucosal layer while endoscopy is ongoing. Different types of tissue are recognisable, and diseases can be diagnosed immediately, facilitating early identification of intraepithelial neoplasia. Analysis of *in vivo* microarchitecture may be helpful in targeting biopsies to relevant areas (Neumann *et al.* 2011). In current diagnostic work-up, however, the role of chromoendoscopy and of CLE is insignificant.

4.4 Imaging techniques

For assessing the small intestine, the current imaging standards are computed tomography (CT) and magnetic resonance (MR). Both techniques can determine disease extension and activity based on wall thickness and increased intravenous contrast enhancement. The extent of these findings, along with the presence of oedema and ulcerations, enables categorization of disease severity (Wold *et al.* 2003). Both CT and MR are also the most precise techniques to detect extraluminal complications such as fistulae and abscesses. Fluoroscopic examinations are clearly inferior to CT or MR in detection of small bowel and extraluminal lesions (Gourtsoyiannis *et al.* 2006). Diagnostic accuracy of CT and of MR in detection of small intestine inflammatory lesions are similar, but for diagnosis of CD specifically in the terminal ileum, both are inferior to ileoscopy (Horsthuis *et al.* 2008). CT has greater availability and is less time-consuming than MR. Imaging examinations need to be repeated, and the IBD population is young, so radiation exposure from CT examination may entail increased health risks. MR should therefore be considered wherever possible. CT and MR examinations of the small intestine require oral luminal contrast for adequate distension. Administration of luminal contrast by enteroclysis allows better distention than does simple oral ingestion. Nasojejunal tube placement leads, however, to radiation exposure and produces discomfort (Negaard *et al.* 2007).

Transabdominal ultrasound (US)—with or without contrast enhancement and the Doppler technique—is another non-ionizing imaging technique providing information on disease activity, in particular for CD limited to the ileum (Fraquelli *et al.* 2005). US is a valuable, widely available, and inexpensive tool to assess the site and extent of inflammation and possible complications, but difficulty of visualization of deep bowel segments and high interobserver variability are significant drawbacks.

4.5 Histology

Analysis of a full ileocolonoscopy biopsy series obtained from all segments of the colon (right colon, transverse colon, left colon and sigmoid, and rectum) and the ileum produces the most reliable diagnosis of CD (Van Assche *et al.* 2010a). Samples preferably come both from areas involved in the disease and from uninvolved areas. Histological examination is routine for IBD diagnosis and is helpful in histological distinction between UC and CD. In UC, inflammation is limited to the colon and is superficial, whereas in CD it is generally transmural, multifocal, and may contain granulomas. Focal (discontinuous or segmental) chronic and patchy inflammation, focal crypt irregularity, and granulomas unrelated to crypt injury are the most accepted microscopic features which allow CD diagnosis. The presence of granulomas is also the central histologic criterion among the Lennard-Jones criteria (Lennard-Jones and Shivananda 1997). The transmural character of CD inflammation can be identified only when surgical samples are available. Other microscopic features detectable in surgical specimens of CD patients are aggregated inflammatory pattern, transmural lymphoid hyperplasia, submucosal thickening, fissures, sarcoid granulomas, abnormalities of the enteric nervous system, and relatively normal epithelial mucin preservation (Van Assche *et al.* 2010a). Distinguishing between CD and intestinal tuberculosis is a diagnostic challenge, as they present analogous histological features, in addition to overlapping clinical, radiological, and endoscopic features (Kim *et al.* 2011). As anti-TNF therapy is associated with a higher incidence of tuberculosis with extraintestinal and disseminated infection, the recommendation is that patients with a suspicion of infection are thoroughly investigated before start of that therapy (Gardam *et al.* 2003).

5 Treatment

Treatment of active CD requires recognition of disease activity, localization (ileal, ileocolonic, colonic, or other), and behaviour (inflammatory, stricturing, or fistulating). Even in cases with mild disease, leaving patients without treatment is seldom an option. As smoking cessation is associated with a 65% reduction in risk for relapse, stopping smoking should be encouraged (Cosnes *et al.* 2001).

5.1 Medical therapy

Before the era of corticosteroids, IBD was a fatal disease for a great proportion of patients; no other medication has had such a great impact on outcome (Truelove and Witts 1955, Malchow *et al.* 1984). Despite the development of more potent drugs against CD during recent decades, the disease is still considered incurable and in many patients it leads to surgery and disability. Medical therapy for CD can be divided into therapy aimed at induction and at maintenance of remission (**Table 3**).

Table 3. Conventional medical therapy in Crohn's disease.

Medical therapy	Notable	References
Corticosteroids: predniso(lo)ne methyl prednisolone budesonide	Good initial clinical response in most patients, but 50% either fail to respond or become steroid-dependent at one year. Only one-third achieve mucosal healing. No role in maintaining remission.	Summers <i>et al.</i> 1979 Modigliani <i>et al.</i> 1990 Faubion <i>et al.</i> 2001 Irving <i>et al.</i> 2007
Mesalazine	Limited benefit in induction and maintenance of remission. Possible benefit in postoperative treatment of small intestinal resection	Hanauer & Stromberg 2004 Van Assche <i>et al.</i> 2010b
Sulphasalazine	Can induce clinical remission in mildly active colonic disease associated with arthropathy. Limited use due to frequent intolerance.	Summers <i>et al.</i> 1979
Antibiotics ciprofloxacin metronidazole	Not first-line therapy. May in combination with azathioprine prevent postoperative recurrence. Appropriate in infectious complications, perineal disease, and in bacterial overgrowth.	Sutherland <i>et al.</i> 1991 D'Haens <i>et al.</i> 2008 Dignass <i>et al.</i> 2010
Thiopurines: azathioprine 6-mercaptopurine	Induces and maintains remission. Lead to mucosal healing in both ileal and colonic disease. Should be started in corticosteroid-dependent or -refractory disease. Unsuitable for rapid induction.	D'Haens <i>et al.</i> 1997 D'Haens <i>et al.</i> 1999a Prefontaine <i>et al.</i> 2009 Prefontaine <i>et al.</i> 2010
Methotrexate	Less studied than azathioprine. Mucosal healing seems to occur during intramuscular therapy.	McDonald <i>et al.</i> 2012

5.1.1 Anti-TNF α antibodies

In patients with CD, the proinflammatory cytokine TNF α plays a role in the inflammatory cascade. Anti-TNFs neutralize by several mechanisms this cytokine and thus interrupt the inflammatory cascade. As anti-TNFs are created by biological processes, they are also called biological drugs or more simply biologicals.

Infliximab is an intravenously administered murine-derived chimeric monoclonal TNF α inhibitor antibody of the immunoglobulin G1 subset; it is the most extensively investigated biological drug available for the treatment of IBD. Approximately two-thirds of patients achieve significant clinical improvement, and nearly half maintain clinical remission after one year of maintenance therapy (Hanauer *et al.* 2002). Additionally, infliximab induces – as early as four weeks after initiation of therapy – mucosal healing based on endoscopic evaluation. This has been shown to reduce risk for recurrence, in addition to reducing hospitalization and surgery (D'Haens *et al.* 1999b, Rutgeerts *et al.* 2004, Schnitzler *et al.* 2009). Infliximab is also effective as induction and maintenance therapy for fistulizing CD (Sands *et al.* 2004) and may serve as monotherapy or be useful in combination with other immunomodulating agents, usually given as

induction doses at weeks 0, 2, and 6, followed by maintenance infusions every 8 weeks (Colombel *et al.* 2010).

Adalimumab is a subcutaneously administered immunoglobulin G1 isotype monoclonal human antibody against TNF α . Evidence indicates that adalimumab is effective as a weekly or biweekly dosage for both induction and maintenance of remission (Hanauer *et al.* 2006, Sandborn *et al.* 2007). It also shows efficacy in treatment of fistulizing CD. The EXTEND trial demonstrated complete mucosal healing after 52 weeks in 24% of patients on adalimumab compared to 0% on placebo (Rutgeerts *et al.* 2012). Patients who have developed antibodies against infliximab may still benefit from adalimumab (Feagan *et al.* 2012). No randomised controlled trials that systemically compare in CD the efficacy of adalimumab and infliximab exist, although based on studies with more or less similar study designs and populations, their efficacy has been considered comparable. A recent retrospective study of 200 matched anti-TNF naïve CD patients reported no significant difference in steroid-free response rates or in adverse effects after one and two years (Kestens *et al.* 2013). Because of its retrospective design, that study lacked data on clinical or endoscopic activity.

Certolizumab pegol is a pegylated, subcutaneously administered humanized TNF α -binding Fab fragment (Schreiber *et al.* 2005). Multiple studies have shown its efficacy as similar to that of the other anti-TNF agents, its effects being more pronounced if the patient is anti-TNF naïve. In one randomized, double-blind, placebo-controlled trial of adults with moderate-to-severe CD, those who responded to induction therapy with certolizumab were more likely to maintain their response and sustain remission at 26 weeks with continuous certolizumab than were those switched to placebo (Schreiber *et al.* 2007).

The need is strong for further biological drugs, because when one antibody loses effectiveness, switching to another becomes necessary. Under study are several biological therapies targeted at mechanisms other than blockade of TNF α , including modulation of other cytokines, blockade of T cells, and blockade of inflammatory cell migration and adhesion (Danese 2012).

Natalizumab, a blocker of α 4-integrin, has shown promising results in CD treatment, but severe adverse effects such as reactivation of a human polyomavirus leading to progressive multifocal leukoencephalopathy limit its use (MacDonald and McDonald 2007). Vedolizumab is a fully humanized α 4 β 7 integrin antibody that has passed a number of phase-III clinical trials. In clinically moderate to severe CD it has been more efficient than placebo in inducing and maintaining clinical remission. As vedolizumab modulates gut but not brain lymphocyte migration, it is at least theoretically less likely than natalizumab to cause progressive multifocal leukoencephalopathy (Sandborn *et al.* 2013c). Apilimod is an inhibitor of the transcription of IL-12 and IL-23, whereas

ustekinumab and briakinumab both target the p40 subunit common to IL-12 and IL-23. As trials investigating these drugs are still in phases I and II, their long-term effects are unclear. However, results indicate that ustekinumab might be useful in patients who have failed to respond to anti-TNF therapy (Sandborn *et al.* 2012). Golimumab is a TNF α -blocking monoclonal antibody newly approved for treatment of UC. Its advantages compared with adalimumab and infliximab are its once-monthly dosing by either intravenous or subcutaneous administration (Sandborn *et al.* 2013a, b). It has not yet advanced into CD trials.

5.2 Surgery

Although surgery in CD should be limited to complications of the disease such as strictures and fistulae, ultimately, a large majority, up to 70%, despite optimized medical therapy, will need surgery. Furthermore, up to 40% will need secondary surgery because of disease recurrence (Bernell *et al.* 2000). Despite its revolutionizing effect on CD treatment, evidence is still limited as to anti-TNF impact on need for surgery. Population surveys during the last two decades have shown inconsistent results, with both declining trends in and no changes in need for surgery (Lazarev *et al.* 2010, Nguyen *et al.* 2011). Subgroup analyses of anti-TNF-responding patients seem to suggest a reduction in the need for surgery at a median follow-up of up to three years (Feagan *et al.* 2008). The short follow-up and exclusion of patients with imminent surgical need could, however, cause bias. CD patients in northern Europe seem more likely to undergo surgery than in southern Europe, suggesting a north-south disease-severity gradient (Wolters *et al.* 2007). To preserve bowel function and minimize risk for intestinal failure, a more conservative surgical approach has been adopted during recent decades. In cases with medical intractability, internal fistulae, abscesses, symptomatic bowel obstruction, severe bleeding, toxic dilatation, or acute perforation, however, surgical resection inevitably becomes necessary (Greenstein *et al.* 1988). Patients with perianal or rectovaginal fistulae often need a combination of surgery and medical treatment. In the surgical management of small bowel CD, strictureplasty plays a central role. Often considered for strictureplasty are isolated strictures under 10 cm in length. A majority of patients achieve symptomatic relief, with secondary surgery rates of between 34 and 44% during a seven-year follow-up (Larson and Pemberton 2004).

5.3 Nutritional therapy

Unlike the management of CD in paediatric and adolescent patients, no placebo-controlled trials involve nutritional therapy for active CD in adult patients. In one Cochrane systematic review, however, elemental or polymeric diets were less effective than corticosteroids in inducing remission (Zachos *et al.* 2001). Enteral therapy is regarded as appropriate only for adjunctive treatment to support nutrition, not for

primary therapy in active CD (Dignass *et al.* 2010). Omega (ω)-3 fatty acids may have anti-inflammatory effects by reducing production of leukotriene B4. Due to the heterogeneous study data, however, the efficacy of ω -3 fatty acids in maintaining remission remains controversial (Turner *et al.* 2009). Data in ten systematically reviewed studies suggest that enteral nutrition as a complement to an ordinary diet may be useful for maintaining remission in patients with CD, although the evidence level is low (Yamamoto *et al.* 2010).

6 Assessment of disease activity

In clinical practice, disease activity assessment relies on clinical history and a combination of clinical, laboratory, endoscopic, and radiological findings. Need for standardisation and quantification of disease severity in clinical trials has led to development of several disease activity indices based on findings or symptoms or their combinations. **Table 4** presents a summary of those assessment methods of clinical activity most commonly used in both clinical practice and trials.

Table 4. Crohn’s disease activity assessment in clinical practice and clinical trials

Assessment	Feature indicating active CD	Commonly used indices
Symptoms and findings	Diarrhoea, bloody stools, abdominal pain, fever, arthralgia, weight loss	CDAI, HBI, PDAI
Blood tests	Elevated CRP, elevated ESR, anaemia, elevated platelet count, low albumin	ESR, blood count, and albumin included in several indices
Faecal markers	Elevated calprotectin, elevated lactoferrin	
Endoscopy	Mucosal ulcerations, inflammation, strictures	CDEIS, SES-CD, Rutgeerts score
Imaging	Fistulae, abscesses, thickened bowel wall, strictures, abdominal lymphadenopathy	

CD: Crohn’s disease; CDAI: Crohn’s disease activity index; HBI: Harvey-Bradshaw index; PDAI: Perianal disease activity index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; CDEIS: Crohn’s disease endoscopic index of severity; SES-CD: simple endoscopic score for Crohn’s disease.

Data from Sostegni R, Daperno M, Scaglione N et al. Review article: Crohn’s disease: monitoring disease activity. Aliment Pharmacol Ther 2003;17:11-17 and Vilela EG, Torres HO, Martins FP et al. Evaluation of inflammatory activity in Crohn’s disease and ulcerative colitis. World J Gastroenterol 2012;18:872–881.

6.1 Clinical activity

In clinical trials, the score most commonly used is the Crohn's disease activity index (CDAI), which comprises one serological and seven clinical variables (**Table 5**), with scores ranging between 0 and approximately 650 (Best *et al.* 1976). A CDAI <150 has defined clinically inactive disease and >450 severe disease. Some investigators have arbitrarily further labeled CDAI scores of 150 to 219 as mildly, and 220 to 450 as moderately active disease (Sostegni *et al.* 2003). One definition for clinical response is a reduction of ≥ 100 points in the CDAI, although some clinical trials have defined response as a reduction of ≥ 70 points (Van Assche *et al.* 2010a). The CDAI score is infrequent in everyday clinical work because of its complex and time-consuming calculation and the need for a seven-day diary of symptoms. Although it has seemed to perform quite well recently in a postoperative setting, it is unsuitable for use as a primary outcome measure in patients with a history of extensive surgery (Walters *et al.* 2011). Further, it is unreliable in patients with a mainly fistulating and stricturing disease. An additional feature of the CDAI score is the considerable weight given for scores on "general well-being" and "intensity of abdominal pain," which are completely subjective (Sostegni *et al.* 2003). Correlation of the CDAI with ileocolonoscopy findings is weak, and the CDAI underestimates endoscopically determined inflammatory activity (Cellier *et al.* 1994, Sipponen *et al.* 2008a, c). For scoring of clinical disease activity of children and adolescents, we have a paediatric Crohn's disease activity index (PCDAI) (Hyams *et al.* 1991).

Table 5. Crohn's disease activity index (CDAI)

Variable	Description	Weighting factor
Number of liquid stools	sum of 7-day numbers	2
Abdominal pain	sum of 7-day scores, subjectively rated 0–3: none=0, mild=1, moderate=2, severe=3	5
General well-being	sum of 7-day scores, subjectively rated 0–4: generally well=0, slightly poor=1, poor=2, very poor=3, terrible=4	7
Extraintestinal features	number of listed features: arthritis/arthralgia, iritis/uveitis, erythema nodosum, pyoderma gangraenosum, aphthous stomatitis, anal fissure/fistula/abscess, fever >37.8°C	20
Antidiarrhoeal medication	use in the previous 7 days: no=0, yes=1	30
Abdominal mass	assessed by palpation: no=0, questionable=2, definite=5	10
Haematocrit (Hct)	women: 42–observed Hct; men: 47–observed Hct	6
Body weight	ideal/observed ratio $[1 - (\text{ideal}/\text{observed}) \times 100]$ (not below –10)	1

Best WR, Beckett JM, Singleton JW, Kern F Jr. *Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology* 1976;70:439-444. Adapted from a figure with permission from Elsevier

Unlike the CDAI, the Harvey-Bradshaw index (HBI) notes only symptoms and signs over the preceding 24 hours (**Table 6**). It is based on five clinical variables: general well-being, graded from 0 to 4 points, abdominal pain and palpable abdominal mass, each graded from 0 to 3 points, number of liquid stools per day, and complications/extraintestinal features, each graded as one point (Harvey and Bradshaw 1980). It has been suggested that HBI scores ≤ 4 or < 4 indicate clinical remission (Best 2006, Vermeire *et al.* 2010).

Table 6. Harvey-Bradshaw index (HBI)	
Variable	Description
General well-being	very well=0, slightly below par=1, poor=2, very poor=3, terrible=4
Abdominal pain	none=0, mild=1, moderate=2, severe=3
Number of liquid stools per day	
Complications	arthralgia, uveitis, erythema nodosum, pyoderma gangraenosum, aphthous ulcers, anal fissure, new fistula, abscess (score 1 per item)
Palpable abdominal mass	no=0, dubious=1, definite=2, definite and tender=3
<i>Harvey RF, Bradshaw JM. A simple index of Crohn's disease activity. Lancet 1980;315:514. Reprinted with permission from Elsevier.</i>	

Both the Oxford Index, based on one laboratory variable and nine clinical variables, and the Cape Town index, originally based on one laboratory variable and nine clinical variables, correlate with the CDAI (Myren *et al.* 1984, Wright *et al.* 1985, Sostegni *et al.* 2003). Because the major contribution of subjective variables to the CDAI has attracted criticism, researchers have attempted to develop disease activity indices on objective grounds only. One instrument that eliminates subjective criteria is the van Hees or Dutch index; it is made up of two laboratory variables, of which serum albumin contributes most, and seven clinical features from patient history or physical examination (van Hees *et al.* 1980). Although the correlation between the van Hees index and the CDAI is poor, both seem to be predictive of CD exacerbations (Wright *et al.* 1985). The CDAI describes poorly the activity of perianal and fistulizing CD; the perianal disease activity index (PDAI) currently represents the gold standard for evaluating perianal disease severity (Irvine 1995, Sostegni *et al.* 2003). Recently, the short CDAI was developed and validated (Thia *et al.* 2011). Of the eight variables in the CDAI, the short version includes only three that are clinical self-reported symptom variables. Though it shows a strong correlation with the CDAI, its self-reporting of subjective symptoms and well-being, means that it has disadvantages similar to those of the original index (**Table 7**).

Table 7. Clinical activity indices for Crohn's disease.

Clinical index	Reference	Variables	Remarks
CDAI	Best <i>et al.</i> 1976	7 clinical, 1 laboratory: diarrhoea frequency, abdominal pain, general well-being, use of antidiarrhoeal medications, abdominal mass, extraintestinal features, haematocrit, weight	Most used in clinical trials, time-consuming for clinical practice, necessitates a 7-day diary
HBI	Harvey and Bradshaw 1980	5 clinical: diarrhoea frequency, abdominal pain, general well-being, abdominal mass, extraintestinal features	Only clinical variables, no need for diary
Oxford Index	Myren <i>et al.</i> 1984	9 clinical, 1 laboratory: abdominal pain, diarrhoea/blood and mucus in stool, perianal involvement, fistulae, other complications, abdominal mass, tenderness, wasting, temperature, haemoglobin	
Cape Town index	Wright <i>et al.</i> 1985	9 clinical, 1-2 laboratory: abdominal pain, stool consistency, well-being, complications (perianal or systemic), fever, abdominal mass, weight, temperature, haemoglobin	ESR later added as a modification
PCDAI	Hyams <i>et al.</i> 1991	8 clinical, 3 laboratory: abdominal pain, diarrhoea, general well-being, weight, height, abdominal findings mass/tenderness, perirectal disease, extraintestinal manifestations, haematocrit, ESR, albumin	For paediatric use
van Hees index (Dutch index)	van Hees <i>et al.</i> 1980	7 clinical, 2 laboratory: body mass index, abdominal mass, sex, temperature, stool consistency, previous resection, extraintestinal manifestations, albumin, ESR	Albumin plays an important role
PDAI	Irvine 1995	5 clinical: discharge of fistulae, pain/restriction of activities, restriction of sexual activity, type of perianal disease, degree of induration	For assessment of perianal Crohn's disease
Short CDAI	Thia <i>et al.</i> 2011	3 clinical: abdominal pain, diarrhoea frequency, general well-being.	Necessitates a 7-day diary

CDAI: Crohn's disease activity index; HBI: Harvey-Bradshaw index; PCDAI: paediatric Crohn's disease activity index; PDAI: perianal disease activity index; ESR: erythrocyte sedimentation rate

6.2 Endoscopic activity and mucosal healing

In patients with long-standing chronic ileocolonic CD, correct diagnosis requires clear-cut indications for endoscopy, assessment of disease activity and extension, dilation of strictures, and surveillance (Hommes and van Deventer 2004). Follow-up endoscopies are required when disease activity or disease location is uncertain. In CD, blood tests and symptoms do not necessarily correlate with endoscopic disease activity; intestinal inflammation can occur in patients free from symptoms (Cellier *et al.* 1994). Ileocolonoscopy has, however, several drawbacks: it is time-consuming and expensive, it requires bowel preparation, and most patients consider it unpleasant.

Since the 1960s, clinical studies on UC have suggested a more favourable outcome after a corticosteroid course in UC patients achieving clinical and endoscopic remission than in those achieving only clinical remission. Up until the late 1990s, studies reported no such correlation in CD patients (Modigliani *et al.* 1990). The introduction of anti-TNF therapy completely changed investigators' and clinicians' attitudes towards mucosal healing; healing of the mucosa for the first time became possible. Since then, constantly growing interest in mucosal healing has revealed its clinical importance as a predictive marker of favourable outcome. Now, assessment of mucosal healing during therapy has become essential for clinical practice and for evaluation of response in clinical trials (Hommes and van Deventer 2004, Rutgeerts *et al.* 2007).

6.2.1 Anti-TNF therapy and mucosal healing

Schnitzler and coworkers (2009) analysed retrospectively for a median of almost five years 214 CD patients who had undergone endoscopy before start of infliximab therapy. Scheduled infliximab therapy led to mucosal healing that was associated with the best long-term outcome; nearly 80% showed sustained clinical benefit from infliximab until the end of the study. Those achieving mucosal healing underwent significantly less surgery and needed less hospital treatment than did those with active disease seen in the follow-up endoscopy done approximately seven months after the start of therapy. In IBD, studies and reviews suggest mucosal healing as a therapeutic goal, with absence of mucosal ulcerations serving in most anti-TNF trials as the definition of mucosal healing (Rutgeerts *et al.* 2006, 2012, Frøslie *et al.* 2007, Isaacs 2010, Neurath and Travis 2012). The importance of minor changes such as an aphthous ulcer in otherwise healed mucosa remains unclear, however.

Most studies on predictive factors in anti-TNF-treated CD have focused on clinical outcome. We know relatively little about factors predicting mucosal healing during anti-TNF therapy. In 201 Hungarian CD patients treated with adalimumab, Kiss and coworkers (2011) found that low CRP (<10 mg/l) at week 12, clinical remission at week 24, and non-smoking were all associated with endoscopic improvement or healing at one year. They defined clinical remission as CDAI<150, and mucosal healing as the absence of any mucosal lesions or signs of active inflammation. Hébuterne and coworkers (2013)

have described the similarity of endoscopic findings in early endoscopic evaluation and in endoscopy one year after start of anti-TNF. Their open-label MUSIC trial involved 89 CD patients treated with certolizumab pegol for 54 weeks. They defined endoscopic response as a decrease in CDEIS score of more than 5, defined maintenance of endoscopic effect as unaltered CDEIS between weeks 10 and 54, and defined complete endoscopic remission as CDEIS<3. In a subpopulation of 52 patients with 54-week endoscopic data, of the 37 who showed an endoscopic response at 10 weeks, 28 (76%) maintained their response at week 54. Of 7 patients in complete endoscopic remission at week 10, 5 (71%) maintained remission at week 54.

Although the EXTEND trial focused mainly on comparing endoscopic outcome after adalimumab or placebo following a two-week adalimumab induction, its results also suggested that mucosal healing may be more difficult to achieve for patients with more severe ulcerations at baseline (Rutgeerts *et al.* 2012).

Optimal timing for determination of mucosal healing remains unsettled (Neurath and Travis 2012). Recently, the concept of deep remission, defined as a combination of clinical and endoscopic remission, has become recognized as a potential predictive CD marker (Rutgeerts *et al.* 2009, 2012, Hommes *et al.* 2012, Travis *et al.* 2012). During adalimumab therapy, deep remission has been associated with lower health-care costs and a favourable long-term outcome in terms of hospitalizations and quality of life (Colombel *et al.* 2011). The definition of deep remission is, however, still evolving.

6.3 Endoscopic scoring of inflammatory activity

The need for reproducibility and standardisation in the management and follow-up of IBD has led to development of several endoscopic grading scores. Endoscopic scores originally classifying disease activity have also been proposed as means to define mucosal healing (Hommes and van Deventer 2004).

6.3.1 CDEIS

The Crohn's disease endoscopic index of severity (CDEIS) was developed at the end of the 1980s by the French Groupe d'Etude des Affections Inflammatoires Digestives (GETAID) (Mary and Modigliani 1989). The CDEIS is validated in terms of reproducibility and global endoscopic evaluation of lesion severity and has become the gold standard for assessment of endoscopic activity in CD (Sostegni *et al.* 2003). Calculation of the CDEIS requires considering the colon and the terminal ileum as comprising five segments: (1) rectum, (2) left colon and sigmoid, (3) transverse colon, (4) right colon, and (5) ileum (**Table 8**).

Table 8. Crohn's disease endoscopic index of severity (CDEIS)

	Rectum		Sigmoid and left colon		Transverse colon		Right colon		Ileum		
Deep ulceration quote 12 if present in the segment, 0 if absent	—	+	—	+	—	+	—	+	—	=	Total 1
Superficial ulceration quote 12 if present in the segment, 0 if absent	—	+	—	+	—	+	—	+	—	=	Total 2
Surface involved by disease measured in cm*	—	+	—	+	—	+	—	+	—	=	Total 3
Ulcerated surface measured in cm*	—	+	—	+	—	+	—	+	—	=	Total 4
Total 1 + Total 2 + Total 3 + Total 4 =											Total A
Number (<i>n</i>) of segments totally or partially explored (1–5)											<i>n</i>
Total A divided by <i>n</i> =											Total B
Quote 3 if ulcerated stenosis anywhere, 0 if not =											C
Quote 3 if non ulcerated stenosis anywhere, 0 if not =											D
Total B + C + D =											CDEIS

*For partially explored segments and for the ileum, the 10 cm linear scale represents the surface effectively explored

Mary JY, Modigliani R. Development and validation of an endoscopic index of the severity for Crohn's disease: a prospective multicentre study. Groupe d'Études Thérapeutiques des Affections Inflammatoires du Tube Digestif (GETAID). Gut 1989;30:983-989. Reproduced with permission from BMJ Publishing Group Ltd.

From each segment, the presence of nine mucosal lesion types would be recorded: (1) pseudopolyp, (2) healed ulceration, (3) erythema (plaques, bands, or diffuse), (4) swollen mucosa, (5) aphthous ulceration, (6) superficial or shallow ulceration, (7) deep ulceration, (8) non-ulcerated stenosis, and (9) ulcerated stenosis. The percentage of segmental surfaces involving the disease and ulcerations are posited on a 10-cm analogue scale between 0 and 10 (no lesion=0, lesions or ulcerations involving 100% of the segment=10). For the terminal ileum and for those colonic segments only partly explored, the 10-cm scale represents the area actually seen. The CDEIS can range between 0 and 44, with higher scores depicting more severe endoscopic activity. Although the CDEIS has served for endoscopic scoring in several studies, threshold values for remission or for mild, moderate, or severe disease, or for significant response are still lacking. After revisiting the endoscopy findings for the validation of the CDEIS, the GETAID study group suggested a cut-off value of 3 or 3.5 for complete mucosal

healing, defined as no lesions or scars, and a rougher cut-off for endoscopic remission set at CDEIS levels of between 6 and 7, defined as no lesions or scars but accepting minor lesions, and for endoscopic response a decrease in the CDEIS of more than 5 (Mary *et al.* 2005, 2006). The CDEIS correlates poorly with clinical activity (Cellier *et al.* 1994).

6.3.2 SES-CD

The time-consuming and complex structure of the CDEIS has prevented it from becoming a tool in everyday clinical practice. To simplify endoscopic assessment of inflammatory activity in CD, the simple endoscopic score for Crohn's disease (SES-CD) was developed and validated nearly ten years ago. Its construction and validation is based on correlations with the CDEIS and, to a lesser extent, the CDAI (Daperno *et al.* 2004). The SES-CD shows a strong correlation with the CDEIS and is easier and quicker to calculate. It is based on four variables scored in the same five ileocolonic segments as in the CDEIS (**Table 9**). The ileum is scored for the full extent to which it is examined, but the ileal score specifically excludes the ileocaecal valve or any ileocolonic anastomosis, which are both included in the neighbouring distal segment. Additionally to the ileocaecal valve, the right colon includes the caecum and the ascending colon up to the hepatic flexure. The transverse colon is defined as the segment between the hepatic and splenic flexures. The left colon includes the descending colon and sigmoid colon. The rectum is defined as that portion distal to the rectosigmoid junction. The SES-CD can range from 0 to 60, with higher scores for increased inflammatory activity. No consensus on cut-offs for remission or different stages of inflammatory activity exists. Suggested definitions on endoscopic remission for the SES-CD have been a score of 0–2 and 0–3 (Sipponen *et al.* 2008a, Schoepfer *et al.* 2010). Moskovitz and coworkers (2007) defined remission as an SES-CD score of 0–2, mild inflammation as 3–6, moderate inflammation as 7–15, and severe inflammation as ≥ 16 . Because variables of each segment of the colon are noted separately before calculation of the total score, minor changes covering several segments may give a higher CDEIS or SES-CD. Thus, it seems that both endoscopic scores overestimate colonic disease and underestimate ileal disease, or severe but limited inflammation in one colonic segment (Sipponen *et al.* 2010a).

Table 9. The simple endoscopic score for Crohn's disease (SES-CD)

Variable	Values			
	0	1	2	3
Size of ulcers	none	aphthous ulcers (<0.5 cm)	large ulcers ($0.5\text{--}2.0$ cm)	very large ulcers (>2.0 cm)
Ulcerated surface	none	$<10\%$	$10\text{--}30\%$	$>30\%$
Affected surface	unaffected	$<50\%$	$50\text{--}75\%$	$>75\%$
Presence of narrowing	none	single, can be passed	multiple, can be passed	cannot be passed

Total SES-CD: Sum of the values of each variable and for every examined bowel segment (rectum; left colon and sigmoid; transverse colon; right colon; ileum).

Daperno M, D'Haens G, Van Assche G et al. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. Gastrointest Endosc 2004;60:505-512. Reprinted with permission from Elsevier.

6.3.3 Other scores

Following curative resection of CD, endoscopic assessment reveals signs of inflammatory activity in up to 70% of patients at 6 to 12 months, and the severity of the lesions predicts subsequent clinical course (Rutgeerts *et al.* 1990). The Rutgeerts score, developed in 1990 for assessment of ileal disease, is considered the gold standard for endoscopical post-surgical recurrence evaluation (Sostegni *et al.* 2003). Findings in the ileum are scored in five categories; i0: no lesions occur in the distal ileum; i1: ≤ 5 aphthous lesions; i2: >5 aphthous lesions with normal mucosa between the lesions, or skip areas of larger lesions or lesions restricted to ileocolonic anastomosis; i3: aphthous ileitis with diffusely inflamed mucosa; and i4: diffuse inflammation with large ulcers, nodules, or narrowing (Rutgeerts *et al.* 1990).

Recent studies have used confocal laser endomicroscopy for *in vivo* assessment of mucosal inflammatory activity in IBD. The Crohn's Disease Endomicroscopy Activity Score (CDEAS) is capable of detecting colonic segments without any macroscopic inflammation that show histological and endomicroscopical evidence of inflammation, enabling discrimination between quiescent CD and normal mucosa in healthy controls (Neumann *et al.* 2012). The score represents the first endoscopic index for CD based on *in vivo* histology but still needs validation. Another endomicroscopic grade, the Watson grade, is based on cell shedding seen in endomicroscopy, and enables assessment of local barrier dysfunction *in vivo*. In patients with complete mucosal healing as defined by conventional white light endoscopy, increased cell shedding has been associated with subsequent relapse within 12 months after endomicroscopic examination (Kiesslich *et al.* 2012).

The drawbacks of endoscopic scores are their inability to assess transmural injury, the penetrating nature of the disease, signs of progression, and structural damage. For example, the clinical activity scores and the endoscopic scores can be similar both in CD patients with recent disease onset who are naïve to treatment, and in patients with a long history of disease who have extensive, irreversible bowel damage from progressive inflammation or previous bowel resection. Therefore, the concept of cumulative bowel damage has evolved, and it has been included in a newly developed index called the Lémann score, or the Crohn's disease digestive damage score (Pariente *et al.* 2011). This score aims to identify CD patients at risk for rapid damage progression who would benefit from early introduction of immunosuppressive or anti-TNF therapy. It is a complex score evaluating strictures and penetrating lesions as well as surgical resection or bypass of the bowel in the whole GI tract. Additionally to medical history and conventional endoscopy, it requires imaging for assessment of transmural damage. The Lémann score ranges from 0 (no inflammation, no damage) to a theoretical value of 10 (complete resection of the GI tract).

6.4 Histological activity

Several clinical drug trials have shown that medical treatment can alter mucosal histology, promoting healing and normalisation of the mucosa (D'Haens *et al.* 1997, 1999a, b, Geboes *et al.* 2005). However, due to the patchy character of the disease, including sample error and the fact that the terminal ileum may be the only area affected, no general agreement exists among experts as to the value of microscopy in assessing CD activity (Van Assche *et al.* 2010a). Assessment of histologic disease activity cannot therefore in general be recommended as a treatment endpoint in clinical trials (Sandborn *et al.* 2002).

6.5 Blood tests

In IBD, anaemia, elevated white blood cells, and increased platelet count are common but nonspecific laboratory findings (Cellier *et al.* 1994). ESR indirectly measures acute-phase plasma protein concentrations. In addition to inflammation, any condition that elevates fibrinogen: pregnancy, diabetes mellitus, end-stage renal failure, heart disease, or malignancy, may also elevate the ESR. ESR is slowly responsive to changes in inflammatory status. In CD, ESR appears to rise with increasing inflammatory activity in colonic disease, but fails to reflect disease activity of the small intestine (Sachar *et al.* 1990, Desai *et al.* 2007).

CRP is an acute-phase protein produced by the liver in response to tissue damage due to inflammation, infection, or injury; high CRP level indicates active disease or some bacterial complication. CRP, the most studied of all laboratory markers, has exhibited the best overall performance. Regrettably, its correlation with CD activity has been

inconsistent (Andre *et al.* 1981, Brignola *et al.* 1986, Boirivant *et al.* 1988, Niederau *et al.* 1997). A proportion of patients with mildly active disease seem to systemically present with low CRP values, which some suggest is a consequence of genetic polymorphism (Carlson *et al.* 2005). High sensitivity CRP (hsCRP) may be a more sensitive method of detecting low-grade inflammatory luminal disease than is standard CRP, but more data are necessary before hsCRP can be implemented in routine clinical use (Jones *et al.* 2008). In CD, albumin correlates inversely with clinical and endoscopic activity. Hypoalbuminaemia may be a consequence of protein loss from the inflamed gut or may result from malnutrition secondary to inadequate protein intake or malabsorption (Modigliani *et al.* 1990, Vermeire *et al.* 2006).

6.5.1 Cytokines and serological biomarkers

As an immunologic response, TNF α is primarily produced by activated macrophages and monocytes. Serum TNF α is often increased in IBD, but as the serum concentrations are not consistently elevated, their value as surrogate markers of IBD activity is limited (Desai *et al.* 2007). Elevated levels of several other serum cytokines and cytokine receptors such as IL-1 β , IL-2, IL-2R, IL-6, IFN- γ , IL-8, IL-23, IL-27, and IL-15 have been apparent in IBD, but these seem to have limited utility as non-invasive markers of disease activity; despite active IBD serum levels frequently remaining low (Reimund *et al.* 1996, Reinisch *et al.* 1999, Desai *et al.* 2007).

Because ASCA levels are fairly stable over time, these markers are of limited value in monitoring CD activity. During anti-TNF therapy, antibody responses in most CD patients to ASCA, pANCA, anti-I2, and anti-ompC seem also to remain unchanged (Landers *et al.* 2002, Papp *et al.* 2007).

6.5.2 Radio-labelled neutrophils

White-cell scans with radio-labelled leukocytes or granulocytes can be helpful in detecting acute inflammation. Abdominal scintigraphy and four-day faecal collection of ¹¹¹Indium-labeled granulocytes is a specific, sensitive, and quantitative method that enables intestinal inflammatory activity assessment. In CD, this technique has been suggested as the gold standard for assessing intestinal inflammation (Saverymuttu *et al.* 1985, Gaya and Mackenzie 2002). In CD patients free from symptoms, it may also detect subclinical mucosal inflammation (Saverymuttu 1986). Because this procedure requires special labelling facilities, it is expensive; in addition, it exposes patients to radiation, and its use in clinical practice is limited.

6.6 Intestinal permeability tests

In CD, intestinal permeability is increased and correlates with inflammatory changes in the small bowel mucosa. It can be assessed non-invasively by measurement of the

urinary secretion of orally administered test substances. Two such substances based on differing renal excretion rates, when combined, give a specific index of intestinal permeability. Most standard test substances comprise a combination of a disaccharide such as lactulose and a monosaccharide (L-rhamnose or mannitol). Other substances include ^{51}Cr -ethylenediamine tetra-acetic acid (^{51}Cr -EDTA), ^{99}Tc -diethylenetriamine penta-acetic acid (^{99}Tc -DPTA), polyethylene glycol (PEG), and iohexol (Bjarnason *et al.* 1995, Halme *et al.* 2000). Lactulose-mannitol and iohexol tests have correlated with endoscopic and clinical CD activity (Halme *et al.* 2000). In CD, increased intestinal permeability during remission demonstrably precedes relapse in CD (Wyatt *et al.* 1997). However, permeability tests are non-specific for IBD, and their diagnostic accuracy in discriminating IBD from non-IBD conditions is inferior to the accuracy of faecal inflammatory markers (Canani *et al.* 2006).

6.7 Faecal tests

Active bowel inflammation is associated with leukocyte infiltration and the production of acute-phase proteins in the mucosa. Because the faecal stream is in direct contact with the intestinal mucosa, hypothetically it should contain specific markers of mucosal disease, ones consistent with the presence and severity of inflammation. Potential faecal biomarkers include faecal excretion of leukocytes, leukocyte products, and serum proteins (Desai *et al.* 2007). The instability of inflammatory markers in the stool has traditionally led to difficulty in accurately assessing inflammatory products in the stool. The presence of faecal white cells—after exclusion of infection—may prove a useful indicator of gut inflammation. The accuracy of this marker depends, however, on rapid examination of the stool sample before degradation of white cells by gut bacteria. Levels of α -1-antitrypsin and proteins released from neutrophils such as myeloperoxidase have also served as inflammatory markers for IBD, but their inadequate accuracy in distinguishing active IBD from healthy controls has prevented their use in clinical practice. Furthermore, comparison of faecal excretion of α -1-antitrypsin with that of ^{111}In -labelled leukocytes has showed an inconsistent correlation (Fischbach *et al.* 1987, Crama-Bohbouth *et al.* 1989).

Several of the S100-family proteins correlate well with intestinal inflammation, particularly faecal calprotectin, which has a positive predictive value of 85 to 90% in distinguishing IBD from IBS (Tibble *et al.* 2000a, Kane *et al.* 2003, D'Inca *et al.* 2007). Their concentrations in stool also correlate with both endoscopic and histologic disease activity in patients with IBD, so the potential exists to replace endoscopy with stool tests to assess mucosal healing during medical therapy and also to predict probability of relapse. However, with their small population sizes and differing definitions of mucosal healing, studies on faecal biomarkers have been unable to define clear-cut points for mucosal healing (Lewis 2011).

6.7.1 Calprotectin

Calprotectin, a heterocomplex of S100A8 and S100A9 with a molecular mass of 36.5 kDa, is a calcium- and zinc-binding protein derived predominantly from neutrophils, and to a lesser extent, from monocytes and reactive macrophages. It comprises up to 60% of the cytosolic protein in human neutrophils (Fagerhol *et al.* 1980). Due to its good resistance to bacterial degradation, calprotectin shows excellent stability in faeces. In IBD, it correlates strongly with excretion of ¹¹¹indium-labeled granulocytes and has also repeatedly correlated with both endoscopic and histological findings. A normal calprotectin concentration has been a useful surrogate marker for endoscopically and histologically inactive disease (Roseth *et al.* 1999, Jones *et al.* 2008, Sipponen *et al.* 2008a, 2010b, Schoepfer *et al.* 2010).

Calprotectin can be quantified from faeces by several different enzyme-linked immunosorbent assays (ELISAs). The original quantitative ELISA developed by Roseth and coworkers (1992) was improved in 2000, when its units were changed from mg/l to µg/g (Tibble *et al.* 2000a). The ELISA test is the most widely used measure of faecal calprotectin concentration. In one study comparing commercial quantitative ELISAs, only small differences were detectable, and all assays tested were considered suitable for routine laboratory measurement of faecal calprotectin (Whitehead *et al.* 2013). Limitations of the ELISA test are that it is time-consuming and requires a laboratory and trained personnel. In addition, collection of multiple samples is necessary to make the running test more cost-effective. As a consequence, rapid point-of-care tests have recently appeared, two of which are a semi-quantitative assay and a rapid quantitative test. These chromatographic immunoassay tests rely on lateral flow assay technology in which the centrifugation step can be omitted (Damms *et al.* 2008). The rapid quantitative test has shown accuracy similar to that of ELISA in detecting endoscopic activity and postoperative recurrence (Lobatón *et al.* 2013).

The remitting and relapsing course of both CD and UC is unpredictable. Estimating relapse risk to enable preventive or early treatment would require an accurate marker. In IBD, one useful predictor of mucosal healing has been low faecal calprotectin level, whereas high calprotectin levels may indicate risk for clinical relapse during clinical remission (Tibble *et al.* 2000b, Roseth *et al.* 2004, Sipponen *et al.* 2008b, Mao *et al.* 2012). Calprotectin as a predictor of long-term outcome in anti-TNF-treated CD is, however, insufficiently investigated. In a study of patients receiving infliximab induction therapy followed by single immunomodulator maintenance therapy, postinduction calprotectin levels failed to predict clinical relapse at one year (Laharie *et al.* 2011). Open questions also exist regarding calprotectin as a marker of intestinal inflammation. Most studies on calprotectin reference values focus on distinguishing IBD from IBS or from healthy controls, but the calprotectin cut-off value for distinguishing IBD from IBS is not necessarily identical to the calprotectin cut-off for distinguishing active IBD from

quiescent IBD. Reference values for an acceptable calprotectin level might even differ depending on type of IBD: one suggestion is that calprotectin concentrations depend on the inflamed bowel segment, with higher concentrations of calprotectin occurring in CD with colonic involvement, but one recent study was unable to confirm this (Sipponen *et al.* 2008c, Schoepfer *et al.* 2010, Jensen *et al.* 2011). Additionally, in a population of patients with mild to moderate clinical activity, considerable intraindividual variance in calprotectin concentrations in stool samples appeared (Moum *et al.* 2010). Thus, despite the frequently used cut-off values of <50 µg/g or <100 µg/g for calprotectin to distinguish between active and inactive CD, the optimal cut-off value for endoscopic remission in CD is still unestablished (von Roon *et al.* 2007, Sipponen *et al.* 2008c, Lewis 2011) (Table 10).

Table 10. Studies investigating the power of faecal calprotectin distinguishing endoscopic remission and endoscopically active disease.

Reference	Patients or endoscopies	Endoscopic definition	Sensi- tivity %	Speci- ficity %	F-Calpro cut-off µg/g
D'Inca <i>et al.</i> 2007	31	Inactive disease: modification of SES-CD=0	81	80	80
Sipponen <i>et al.</i> 2008c	77	Active disease: CDEIS≥3	91	44	50
			81	69	100
			70	92	200
Langhorst <i>et al.</i> 2008	43	No inflammation: No visible inflammation in endoscopy	81	80	48*
Schoepfer <i>et al.</i> 2010	140	Active disease: SES-CD≥4	89	58	50
			89	72	70
D'Haens <i>et al.</i> 2012	87	Remission: CDEIS 0–3	94	62	250
Lobatón <i>et al.</i> 2013	37	Remission: CDEIS 0–3	76	97	274
	111	No ulcers vs. ulcers	68	84	283
F-Calpro: faecal calprotectin					
*µg/ml					

6.7.2 S100A12, lactoferrin, and polymorphonuclear neutrophil elastase

S100A12, also known as calgranulin C, is expressed as a cytoplasmic protein in neutrophils and has pro-inflammatory properties (Foell *et al.* 2003). It activates the nuclear factor-κB signal transduction pathway, upregulating TNFα and further enhancing S100A12 expression (Hofmann *et al.* 1999). These properties are relevant to IBD, with infiltration of S100A12-positive polymorphonuclear cells potentially contributing to the invasion of other leukocytes (Leach and Day 2006). This may suggest that S100A12 contributes to the processes of intestinal inflammation (de Jong *et al.*

2006). S100A12 is evenly distributed in faeces and is stable despite temperature changes for seven days, characteristics suitable for a non-invasive, stool-based disease marker. In predicting small bowel inflammatory changes, S100A12 shows moderate specificity but low sensitivity (Sipponen *et al.* 2012). According to one study, faecal S100A12 correlates better with intestinal inflammation than does faecal calprotectin or other biomarkers. In distinguishing active IBD from IBS, S100A12 sensitivity was 86%, and specificity was 96%, superior to the sensitivity (63%) and specificity (86%) of calprotectin (Kaiser *et al.* 2007). The role of S100A12 as a predictive marker has not yet been established.

Lactoferrin, an iron-binding glycoprotein secreted by most mucosal membranes that interact directly with external pathogens, is detectable in saliva, tears, vaginal secretions, faeces, synovial fluid, and mammalian breast milk. It is a major component of the secondary granules of polymorphonuclear neutrophils, which are a primary component of the acute inflammatory response (Baynes and Bezwoda 1994, Kayazawa *et al.* 2002). In the intestinal lumen, during inflammation, with its influx of neutrophils, faecal lactoferrin levels quickly increase (Desai *et al.* 2007). Having antibacterial activity and being resistant to proteolysis in the faeces, lactoferrin may remain stable in stool for as long as five days compared with seven days for calprotectin (Sugi *et al.* 1996). Several studies indicate the usefulness of measuring lactoferrin in patients with IBD (Kane *et al.* 2003). Although most have reported similar sensitivities and specificities for both lactoferrin and calprotectin in differentiating chronic IBD from IBS, some studies also indicate that lactoferrin's performance would be slightly inferior to that of calprotectin (Silberer *et al.* 2005).

Polymorphonuclear neutrophil elastase (PMN-e) is a neutral proteinase normally stored in the azurophil granules of polymorphonuclear neutrophils but released by activation of these cells as a mediator of inflammation. It has proved its clinical value as a test for pancreatic exocrine dysfunction, but it may also play a role in IBD in assessing disease activity (Poullis *et al.* 2002). In a study both of UC and of CD patients comparing three stool markers with endoscopic findings, CRP and the clinical indices calprotectin, lactoferrin, and PMN-e were all able to differentiate between active IBD and inactive IBD and also to distinguish IBD from IBS. Although the PMN-e levels were significantly higher in active than in inactive IBD, and all three faecal markers were superior to CRP and the CDAI in their diagnostic accuracy, calprotectin seemed to have the highest accuracy for CD (Langhorst *et al.* 2008).

6.8 Combined activity scores

Global assessment of CD activity requires a combination of clinical observation, laboratory and endoscopy findings, and in certain situations, radiological imaging. Attempts to successfully combine different noninvasive laboratory tests and clinical findings and symptoms to assess the presence or absence of endoscopically determined inflammation are rare. Langhorst and coworkers (2008) studied the performance of the

CDAI, CRP, calprotectin, lactoferrin, and PMN-e in discriminating between active and inactive inflammation in 43 CD patients. Inactive inflammation was defined as the complete absence of inflammatory lesions in all observable segments of the colon and terminal ileum. Clinical examination and contrast-enhanced magnetic resonance imaging of the small bowel excluded extraintestinal and small bowel involvement. Their comprehensive activity index was rated positive when it met at least two of three conditions: elevation of at least two stool values, elevated CRP, and elevated CDAI. CRP > 7 mg/l and CDAI > 80 were considered elevated, whereas stool samples were elevated based on optimized cut-off values: calprotectin > 48 µg/ml, lactoferrin > 7.05 µg/ml, PMN-e ≥ 0.062 µg/ml. Sensitivity for this categorical index was 79% and specificity 70%, these values being superior to those of both CRP and the CDAI, but still inferior to calprotectin (sensitivity 82%, specificity 80%).

6.9 Tools for assessing efficacy of anti-TNF therapy in CD

Studies of patient- and disease characteristics predicting a response in anti-TNF-treated CD have generally been based on clinical data (Vermeire *et al.* 2002, Arnott *et al.* 2003, Orlando *et al.* 2012). Despite extensive research on various tests of disease activity in luminal CD, in randomised controlled trials investigating the efficacy of anti-TNF, the primary endpoint by far the most studied has been clinical response or remission (Hanauer *et al.* 2002, Sandborn *et al.* 2007). Mucosal healing as an endpoint has only recently been included in larger studies (Rutgeerts *et al.* 2012). As for CRP, faecal calprotectin, or radiology, studies are limited to the observational, are *post hoc*, or are very small (Sipponen *et al.* 2008b, Kiss *et al.* 2011, Reinisch *et al.* 2012 Van Assche *et al.* 2013) (Table 11).

Table 11. Tools for assessing and predicting response of anti-TNF therapy in luminal Crohn's disease.

Tool	Reference	Variables	Remarks
History	Vermeire <i>et al.</i> 2002, Parsi <i>et al.</i> 2002, Arnott <i>et al.</i> 2003, Orlando <i>et al.</i> 2012	Smoking worsens both clinical and endoscopic response, older age and history of surgery are associated with worse clinical response, isolated colonic disease is associated with better response	Mostly clinical outcome studied
Clinical activity	Hanauer <i>et al.</i> 2002, Sandborn <i>et al.</i> 2007, Kiss <i>et al.</i> 2011,	CDAI <150 (or ≤150) or drop of CDAI >70 or >100	By far the most studied endpoint in anti-TNF trials
Endoscopic findings	Schnitzler <i>et al.</i> 2009, Rutgeerts <i>et al.</i> 2012, Hébuterne <i>et al.</i> 2013	"No ulcers" or CDEIS <3 or decrease of CDEIS >5	No established cut-off in clinical practice
Stool markers	Sipponen <i>et al.</i> 2008b	Decline of faecal calprotectin and lactoferrin levels between baseline and 3 months	No established cut-off in clinical practice
CRP	Kiss <i>et al.</i> 2011, Reinisch <i>et al.</i> 2012	Low CRP <10 at 24 weeks or CRP normalisation at week 14	High baseline levels enhance the probability of maintaining remission
Radiology	Van Assche <i>et al.</i> 2013	Decrease in wall thickening at week 2 or 26	Only 20 patients included
Anti-TNF: anti-tumour necrosis factor- α antibodies; CRP: C-reactive protein; CDAI: Crohn's disease activity index; CDEIS: Crohn's disease endoscopic index of severity			

AIMS OF THE STUDY

Endoscopic resources are limited, and no consensus exists on optimal timing for assessment of anti-TNF therapy response in CD patients. The aims of the present study of patients with active luminal CD were to

1. evaluate the role of endoscopic assessment and non-invasive markers of disease activity in predicting long-term endoscopic response to anti-TNF therapy (Studies I to III).
2. evaluate the power of non-invasive markers of disease activity alone and in combination as a replacement for endoscopy (Study IV).
3. develop a non-invasive combination score of disease activity markers to reduce need for endoscopy (Study IV).

PATIENTS AND METHODS

1 IBD-HOT study

The main aim of the IBD-HOT (Inflammatory Bowel Disease–Health Outcome of Treatment) observational study at the Division of Gastroenterology, Helsinki University Central Hospital, was to assess the use, efficacy, and safety of strong immunosuppressants and anti-TNF in patients with CD and UC. This would allow the construction of a systematic follow-up tool in the form of a register for surveillance of patients with severe disease. The study consisted of two phases: one retrospective, with data on all IBD patients treated with strong immunosuppressants and infliximab between 1999 and the end of 2006, and a prospective three-year observational follow-up study including all IBD patients scheduled for treatment with strong immunosuppressants or with anti-TNF between January 2007 and December 2010. The patients were treated according to standard clinical routines by gastroenterologists at the Division of Gastroenterology; study participation affected neither treatment nor choice of medication. Retrospectively collected data were available for 148 IBD patients and prospectively collected data for 212 IBD patients.

2 Patients

2.1 Retrospective studies (I, III)

2.1.1 Patients in Study I

This study was based on a retrospectively constructed medical database collated from a register of all consecutive patients with IBD who were treated with infliximab between 1999 and the end of 2006. For the study, 71 CD patients (35 female, 49%) fulfilled the following inclusion criteria: infliximab-treated active luminal ileitis, colitis, or ileocolitis, and their data on endoscopy at baseline and 3 months after start of treatment. Exclusion criteria were isolated fistulizing disease, isolated stricturing disease, and upper GI involvement. CD diagnosis was based on standard clinical, endoscopic, radiological, and histological criteria. The Montreal classification served for CD classification; 51 patients had pure inflammatory disease and 20 patients had inflammatory disease complicated with strictures or fistulae (Satsangi *et al.* 2006). All patients presented with moderate to very severe luminal inflammation as determined by endoscopy. Analysis was of the outcome of the first infliximab therapy given for each patient.

2.1.2 Patients in Study III

Study III comprised retrospectively analysed data from 60 IBD patients (34 CD, 26 UC) from both the retrospective and prospective parts of the IBD-HOT study, treated for active luminal disease with anti-TNF (infliximab or adalimumab) between April 2005 and April 2010. All patients had elevated faecal calprotectin levels at baseline and had

data available on calprotectin after induction with anti-TNF. Of the 34 CD and 26 UC patients, 15 and 19, respectively, were female. For induction, patients received either infliximab (26 UC and 16 CD patients, 5 mg/kg at weeks 0, 2, 6, or at weeks 0, 8), or adalimumab (18 CD patients, 160 and 80 mg given at weeks 0 and 2, followed by 40 mg every other week, or 80 mg at week 0, followed by 40 mg every other week for 8–12 weeks). After the induction therapy, anti-TNF was continued in all patients with endoscopic or clinical response as scheduled maintenance therapy for at least one year if no relapse occurred earlier.

2.2 Prospective studies (II, IV)

2.2.1 Patients in Study II

This study included data collected between January 2007 and December 2010 on 42 patients (20 female, 48%) followed up for approximately one year from start of anti-TNF therapy. All patients had endoscopy-verified active, ongoing luminal CD at baseline and received treatment for at least 3 months with anti-TNF. More than half the patients (52%) had a history of bowel surgery due to CD complications. The patients received 3-month induction therapy with either infliximab or adalimumab. Standard induction therapy with adalimumab was 160 and 80 mg given subcutaneously at weeks 0 and 2, followed by 40 mg given every other week, and with infliximab 5 mg/kg given intravenously at weeks 0, 2, and 6. Three months after start of anti-TNF, all patients underwent follow-up endoscopy. Patients with an endoscopic or clinical response continued anti-TNF as maintenance therapy administered as adalimumab 40 mg every other week or infliximab 5 mg/kg every 8 weeks. All patients had data either on endoscopy obtained approximately one year from start of anti-TNF, or had data on bowel surgery performed between 3 months and roughly one year from start of anti-TNF.

2.2.2 Patients in Study IV

Of those 109 patients with endoscopically documented active luminal CD receiving anti-TNF therapy (either infliximab or adalimumab) between January 2007 and December 2010, we identified 64 (32 female, 50%) with data both on the SES-CD and on at least one other non-invasive disease-activity-measuring approach, obtained within 28 days from endoscopy. Only endoscopy examinations with complete data on all existing bowel segments were included; if a stricture impeded the endoscopist in scoring a proximal bowel segment, the endoscopic assessment was excluded. Patients with isolated upper GI disease were also excluded. Because many patients underwent more than one endoscopic assessment, data covered 210 endoscopies. Patients were on anti-TNF therapy during 124 endoscopic assessments.

Table 12 summarises basic data of all four studies performed.

Table 12. Basic data from Studies I-IV

Study	Study nature and main aims	Patients, <i>n</i>	Remarks
I	Retrospective evaluation of early endoscopy as a predictor for long-term endoscopic response	71	Similar construction and objective in Studies I and II
II	Prospective evaluation of early endoscopy as a predictor for long-term endoscopic response	42	
III	Retrospective study of early faecal calprotectin as a predictor for long-term clinical response	60	34 CD patients 26 UC patients
IV	Prospective study of surrogate markers of endoscopic activity	64	210 endoscopies

CD: Crohn's disease; UC: ulcerative colitis

3 Methods

3.1 Endoscopic scoring

In Study I, scoring of CD activity was retrospective. We reassessed the endoscopy reports and their six or more colour prints of the findings. The scoring itself was unblinded, and scores were based on the consensus of two experienced specialists (U.N., U.T.). Inflammatory activity was graded according to mucosal activity in the most-affected area as: 0: remission; 1–2: very mild or mild inflammatory activity (light mucosal erythema or granularity or aphthous inflammation, without ulcerations); 3–4: moderate activity (superficial ulcerations); 5–6: severe or very severe activity (deep ulcerations, with a diameter of under or over 2 cm). The criterion for a positive endoscopic response was—somewhat arbitrarily—a decrease from baseline in endoscopic score of at least two points. As the definition of mucosal healing can range from normal endoscopic findings to light mucosal erythema or granularity without ulcerations (Frøslie *et al.* 2007), it was determined in Study I as a mucosal activity score of 0–2.

In Studies II, III, and IV, CD inflammatory activity was scored according to the SES-CD (Daperno *et al.* 2004). An SES-CD score of 0–2 was defined as remission, 3–6 as mildly active disease, 7–15 as moderately active disease, and ≥ 16 as severely active disease, based on Moskovitz and coworkers (2007). Additionally, in some further calculations, an SES-CD score of 0 represented a normal finding. The terms “mucosal healing” in Study I and “endoscopic remission” in Studies II, III, and IV are interchangeable, even though their definitions vary somewhat. The term “endoscopically inactive disease” in the results section for Studies I and II covers both of those terms.

In Study III, the Mayo endoscopic subscore served for assessment of inflammatory activity in 26 UC patients. Endoscopic findings were graded as normal (0), mild (1), moderate (2), or severe (3); a subscore of 0–1 we defined as remission and a subscore of ≥ 2 as active disease (D'Haens *et al.* 2007).

3.2 Faecal calprotectin and blood tests

Faecal calprotectin was measured by a quantitative enzyme immunoassay (PhiCal Test; Calpro AS, Oslo, Norway), with $<100 \mu\text{g/g}$ of stool considered normal (von Roon *et al.* 2007). Blood count, serum albumin, and CRP were all determined routinely.

3.3 Clinical activity scores

The CDAI served as a measure for clinical disease activity as follows: <150 as clinically inactive disease; 150–219, mildly active disease; 220–449, moderately active disease; ≥ 450 , severe disease (Best *et al.* 1976, 1979, Sandborn *et al.* 2002). Despite a good correlation between CDAI and HBI, conversion of generally approved CDAI cut-off values to the less robust HBI is inexact. As no consensus exists on cut-off values for the HBI, its definitions for clinical remission differed between Studies III and IV: in III, $\text{HBI} < 4$ indicated clinically inactive disease, $\text{HBI} 4\text{--}7$ mildly active disease, $\text{HBI} 8\text{--}16$ moderately active disease, and $\text{HBI} > 16$ severely active disease; whereas in IV, clinical remission was defined as $\text{HBI} \leq 4$ (Harvey and Bradshaw 1980, Best 2006, Vermeire *et al.* 2010).

The 26 UC patients in Study III were clinically assessed with the Mayo clinical subscore (total Mayo score without endoscopy). This subscore grades three clinical variables: frequency of defecation (0–3), amount of blood in stool (0–3), and overall well-being (0–3). Clinical remission was defined as Mayo clinical subscore 0; mildly active disease as 1–3; moderately active disease as 4–6; and severely active disease as ≥ 7 (D'Haens *et al.* 2007).

3.4 Statistics

Continuous variables, expressed as medians and ranges or interquartile ranges (IQR), or as means and standard deviations, were compared by Student's t-test. Correlations between variables were estimated with the two-tailed Spearman's rank order correlation coefficient (r). Receiver operator characteristic (ROC) curves were created to analyze the accuracy of surrogate markers and clinical indices to identify endoscopic remission, and accuracy of SES-CD, CDAI, and calprotectin to predict it. The optimized cut-off level offered the best combination of sensitivity and specificity. Areas under the ROC curves were compared by Delong's method. For Study III, a power calculation was performed. Statistical significance was set at $p < 0.05$. Data were analysed by either NCSS 2004-statistical software (NCSS Statistical Systems, Kaysville, UT, USA) (I), or IBM SPSS Statistics 17–19 software (IBM Corporation, Chicago, IL) (II–IV).

3.5 Ethics

For participation in the studies, all patients gave their informed written consent. The ethics committee of the Helsinki University Central Hospital approved all studies.

RESULTS

1 Predicting long-term endoscopic response

1.1 Endoscopic assessment as a predictor for long-term treatment response

Except for the retrospective nature of Study I and the prospective nature of Study II, these studies had similar structures and objectives. At baseline, all 71 patients in Study I presented with endoscopically determined moderate to very severe luminal inflammation (mucosal activity score 4–6) (**Table 13**). They were divided into two subgroups by indication for infliximab treatment: one subgroup of 51 patients with isolated luminal inflammation and another of 20 patients with luminal inflammation complicated by strictures or fistulae (complicated CD). A clearly higher frequency of smoking in the complicated CD subgroup was the only statistically significant difference in baseline data between subgroups.

Table 13. Baseline data for patients in Study I and II

	Study I <i>n</i> =71	Study II <i>n</i> =42
Sex , male/female, <i>n</i> (%)	36/35 (51/49)	22/20 (52/48)
Age , years, median (range)	32.1 (16.8–73.3)	39.8 (19.1–66.7)
Age at diagnosis , years, median (range)	22 (7–51)	24.5 (14–56)
Duration of disease , years, median (range)	7 (0–30)	11 (1–45)
Age at diagnosis according to Montreal classification , <i>n</i> (%)		
<17 years	12 (17)	7 (17)
17–40 years	54 (76)	28 (67)
>40 years	5 (7)	7 (17)
Localisation of disease , <i>n</i> (%)		
Ileum	13 (18)	6 (14)
Colon	25 (35)	12 (28)
Ileum and colon	33 (46)	24 (57)
Behaviour of disease , <i>n</i> (%)		
Non-stricturing, non-penetrating	51 (72)	14 (33)
Stricturing	10 (14)	11 (26)
Penetrating	10 (14)	17 (40)
Perianal	13 (18)	9 (21)
Current smoking , <i>n</i> (%)	25 (35)	18 (43)
History of anti-TNF therapy , <i>n</i> (%)	-	9 (21)
Laboratory values		
C-reactive protein, mg/l, median (range)	11 (<3–184)	9 (<3–105)
Haemoglobin, g/l, median (range)	128 (93–164)	124 (91–169)

Infliximab treatment usually commenced within one month after baseline endoscopy (median 0.6 months, range 0.0–5.5). At the first follow-up endoscopy at 3 months, patients had received 2 (range 1–3) infliximab infusions. At this point, of the 71 patients, 53 (75%) displayed significant improvement in inflammatory activity as determined by endoscopy, and 32 (45%) had achieved mucosal healing (mucosal activity score 0–2). After the first follow-up endoscopy, further follow-up data were available on 57 patients. Of these 57, 44 (77%) continued infliximab for a period of 2.0 to 15.2 months, receiving a median of 3 infusions (range 1–8). At the second follow-up endoscopy performed at roughly one year after start of infliximab therapy, 34 (60%) had a positive endoscopic response and 30 (53%) presented with mucosal healing.

Study II included 42 patients with endoscopically determined active luminal CD, all treated with anti-TNF (either adalimumab or infliximab) (**Table 13**). All underwent baseline endoscopy before start of anti-TNF. The findings of the 3-month follow-up endoscopy, performed 2.9 months (range 2.2–4.5) from start of anti-TNF, differed significantly from baseline endoscopic findings (baseline SES-CD 14.5, range 4–36, 3-month SES-CD 5.5, range 0–24, $p<0.001$). All 10 patients in 3-month endoscopic remission and of the 32 patients with endoscopically active disease, 23 (72%) continued anti-TNF as maintenance therapy until the one-year follow-up or until they underwent bowel surgery. At the one-year follow-up, of those 33 receiving anti-TNF maintenance therapy, 11 (33%) were in endoscopic remission; additional deep remission, including both endoscopic and clinical remission, was present in 10 patients (30%).

Although one-year endoscopically inactive disease was apparent in patients presenting either with endoscopically inactive or with active disease at the 3-month follow-up, after anti-TNF maintenance therapy, one-year endoscopically inactive disease was significantly more common in those who had presented with inactive disease at three months than in those who had presented with endoscopically active disease (Study I: 90% vs. 33%, $p=0.0001$; Study II: 70% vs. 17%, $p=0.01$) (**Figures 1 and 2**).

Additionally, no patient with endoscopically inactive disease at 3 months developed complications requiring surgery during the follow-up. Four patients in Study I and six in Study II, each lacking any initial endoscopic response, underwent bowel surgery within 12 months from the start of anti-TNF treatment because of complications arising from persisting disease activity.

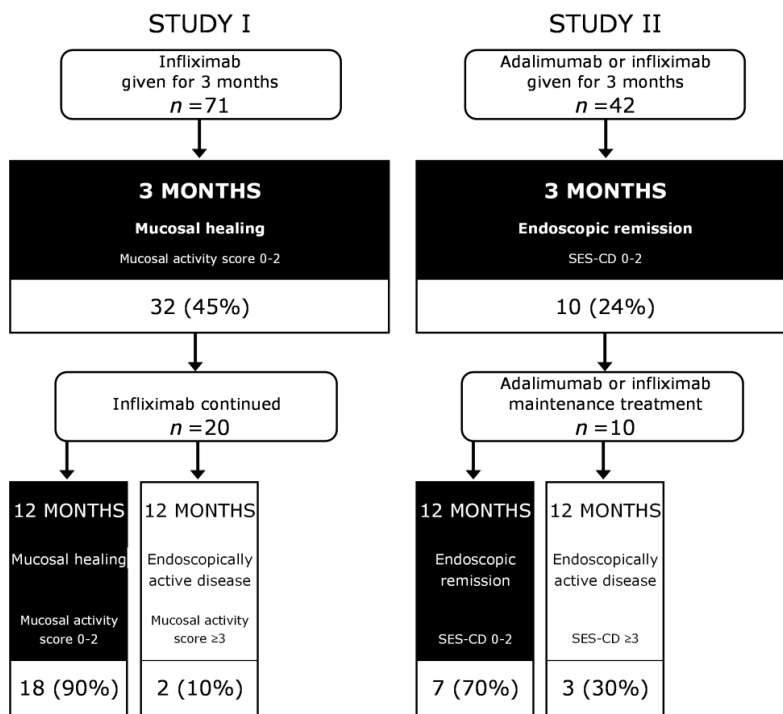


Figure 1. 12-month endoscopic data according to 3-month mucosal healing or endoscopic remission of those 32 patients in Study I continuing infliximab and those 10 patients in Study II continuing either adalimumab or infliximab after 3 months. The mucosal activity score of 0–2 used in Study I implied no inflammatory activity or only very mild inflammation without ulcerations visible in endoscopy. SES-CD: simple endoscopic score for Crohn’s disease. Data adapted from figures from Studies I and II.

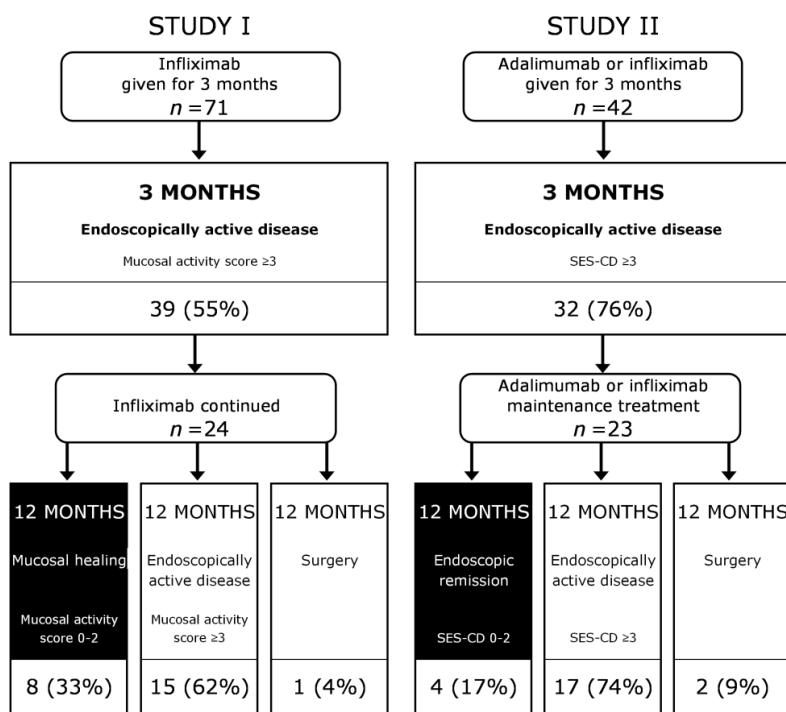


Figure 2. Endoscopic remission, endoscopically active disease, and surgeries at 12 months according to 3-month endoscopy indicating active disease in those 24 patients in Study I who continued infliximab and in those 23 patients in Study II who continued either adalimumab or infliximab after 3 months. The mucosal activity score of 0–2 used in Study I implied no inflammatory activity or only very mild inflammation without ulcerations visible in endoscopy. SES-CD: simple endoscopic score for Crohn’s disease. Data adapted from figures from Studies I and II.

No infections or other adverse effects necessitating discontinuation of anti-TNF therapy nor any deaths occurred during follow-up. In terms of endoscopically determined disease activity, patients in Study I with isolated luminal inflammation appeared to respond better to infliximab therapy than did patients with complicated disease. A positive endoscopic response at the first follow-up was significantly more common in the pure luminal inflammation group than in the complicated CD group (84% vs. 45%, $p=0.003$ at 3 months and 69% vs. 33%, $p=0.02$ at 12 months). Significant differences in mucosal healing, however, emerged between the two subgroups only at 12 months (64% vs. 20%, $p=0.003$). Neither localisation, duration of disease, age at diagnosis, current age, number of infliximab infusions, nor use of concomitant immunosuppressive medication showed any association with the positive or negative endoscopically determined response, nor with mucosal healing.

In Study II, at baseline neither patient nor disease characteristics predicted long-term endoscopic outcome: in a logistic regression analysis of patients in Study II, the 3-month SES-CD proved the only predictor for one-year endoscopic remission with statistical significance ($p=0.03$, odds ratio 0.793, 95% confidence interval 0.644–0.978). A ROC curve analysis for the 3-month SES-CD (using its cut-off value of <3) revealed a 90% sensitivity and 64% specificity to predict one-year endoscopic remission in patients receiving anti-TNF maintenance therapy (**Figure 3**).

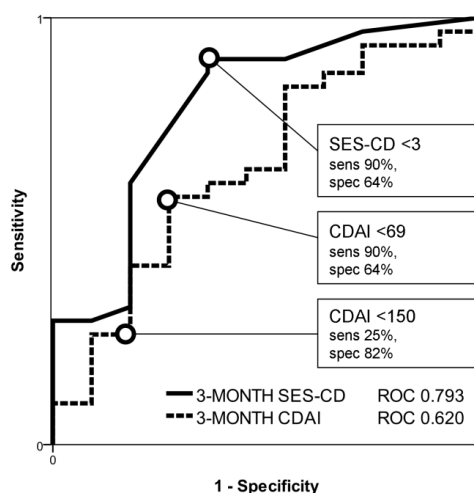


Figure 3. Receiver under the operating characteristic (ROC) curves depicting the SES-CD (AUC 0.793 ± 0.160 , $p=0.004$) and CDAI (AUC 0.620 ± 0.196 , $p=0.25$) at 3 months from start of anti-TNF as prognostic tests for endoscopic remission, defined as SES-CD ≤ 2 , at 12 months, $n=42$. SES-CD: Simple endoscopic score for Crohn's disease; CDAI: Crohn's disease activity index; anti-TNF: anti-tumour necrosis factor- α antibody; AUC: Area under the ROC curve; sens: sensitivity; spec: specificity. Data adapted from a figure from Study II.

1.2 Non-invasive disease-activity assessment as a predictor for long-term treatment response

At baseline, all 60 IBD patients in Study III had elevated faecal calprotectin levels (mean 810 $\mu\text{g/g}$, range 103–12,258 $\mu\text{g/g}$). After induction with anti-TNF, their calprotectin levels had fallen to a mean 97 $\mu\text{g/g}$ (range 0–5,859 $\mu\text{g/g}$, $p<0.001$), and in 31 patients calprotectin had normalized. Of these 31 patients with normal postinduction calprotectin, 27 had a calprotectin decline of more than 75% from baseline. Based on calprotectin levels after anti-TNF induction, the patients were divided into two groups: a normal-postinduction calprotectin group and an elevated-postinduction calprotectin group. Those with the latter who were in clinical remission had a significantly lower calprotectin (median 204 $\mu\text{g/g}$, range 116–670 $\mu\text{g/g}$) than did those with clinically active disease (median 496 $\mu\text{g/g}$, range 123–2,896 $\mu\text{g/g}$, $p=0.025$). At one year, of the original 60 patients, 37 (62%, 27 CD and 10 UC) were in clinical remission, 15 (25%, all UC) had undergone surgery, and 8 (13%, 7 CD and 1 UC) had clinically active disease. Of those 31 patients with a normal postinduction calprotectin, 26 (84%) were in clinical remission, whereas a significantly smaller proportion of the patients with an elevated postinduction calprotectin had achieved clinical remission (11 patients, 38%, $p=0.0002$) (**Figure 4**).

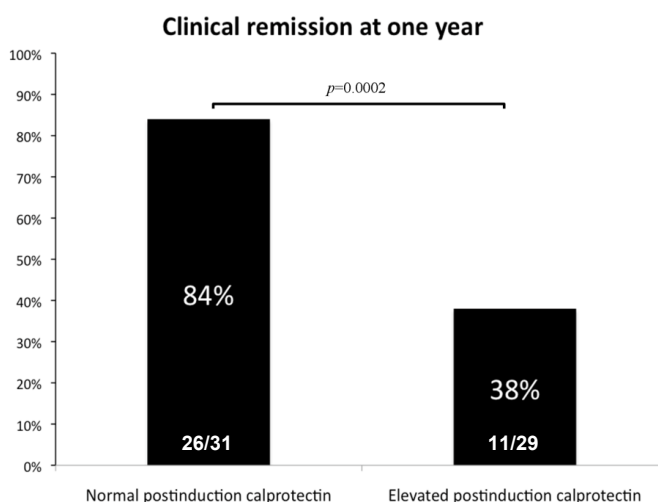


Figure 4. One-year clinical remission in inflammatory bowel disease patients, defined as Harvey-Bradshaw index <4 or Mayo clinical subscore $=0$, according to postinduction calprotectin levels (Study III). Faecal calprotectin <100 $\mu\text{g/g}$: normal; ≥ 100 $\mu\text{g/g}$: elevated. Normal postinduction calprotectin group: 26 patients of 31 in clinical remission at one year; elevated postinduction calprotectin group: 11 patients of 29 in clinical remission at one year.

Of those 27 patients with more than a 75% calprotectin decline at postinduction assessment, 22 (82%) were in clinical remission at one year. At one year, of those 48 patients on maintenance therapy, 25 had available calprotectin concentrations. In these patients, of 17 with a normal postinduction calprotectin, 13 (76%) still showed normal calprotectin, whereas of 8 with an elevated postinduction calprotectin, 4 (50%) showed normal one-year calprotectin. At one year, 38 patients (27 CD and 11 UC) underwent endoscopic reassessment. Endoscopic remission at one year was also more common, although not statistically significant, in those with normal postinduction calprotectin compared to those with elevated postinduction calprotectin (17 of 23, 74% vs. 7 of 15, 47%, $p=0.089$).

With ROC statistics we analysed the power of postinduction calprotectin levels to predict long-term clinical outcome. Calprotectin with a cut-off 139 $\mu\text{g/g}$ showed a sensitivity of 72%, a specificity of 80%, and an area under the ROC curve (AUC) of 0.838 to predict one-year clinical relapse. Because of the small subgroups, no separate cut-offs for CD and UC patients were reasonable to calculate. Additional calculations allowed analysis of the predictive power of calprotectin decline from baseline to postinduction assessment. An 88% calprotectin decline during anti-TNF induction predicted clinical remission at one year with a sensitivity of 87% and specificity of 65% and with an AUC of 0.771.

In Study II, clinical and laboratory data enabled assessment of one-year endoscopic or surgical outcome, based on clinical symptoms and CRP after 3 months of anti-TNF therapy. Neither CRP nor the CDAI (**Figure 3**) at 3 months showed any connection with 12-month endoscopic remission.

2 Non-invasive markers of disease activity as replacement for endoscopy

Of a total of 210 endoscopic assessments at baseline prior to, and 3, 12, 24, and 36 months after start of anti-TNF in the 64 patients in Study IV, no inflammatory lesions at all (SES-CD=0) were detectable in 29 endoscopies (14%), and endoscopy indicated remission (SES-CD \leq 2) in 42 assessments (20%). The proportion of assessments indicating clinical remission according to the CDAI (98 of 141, 69%) and the HBI (100 of 150, 67%) was almost three times as high, and CRP was normal (103 of 209, 49%) nearly twice as often as SES-CD-indicated remission. The proportion of disease activity assessments with a normal calprotectin (37 of 126, 29%) was, on the other hand, nearly identical to the proportion for SES-CD-indicated remission. However, of those 37 disease-activity assessments with normal calprotectin, concurrent active disease was visible in 16 endoscopies (11 with mild, 3 with moderate, and 2 with severe inflammation according to the SES-CD).

Although the endoscopic score showed a correlation with clinical indices and surrogate markers, the SES-CD showed a weaker correlation with both the CDAI ($r=0.40$, $p<0.001$) and HBI ($r=0.32$, $p<0.001$) than the concurrent correlations of SES-CD with CRP ($r=0.56$, $p<0.001$) and calprotectin ($r=0.56$, $p<0.001$). The strongest correlations were between the CDAI and the HBI ($r=0.73$, $p<0.001$). When categorizing disease activity according to the clinical indices into clinical remission, and clinically mild, moderate, and severe disease, statistically significant differences in both the SES-CD and CRP were more difficult to detect. For example, SES-CD scores in the CDAI remission group did not differ from those scores in the CDAI mild-activity group. A corresponding categorization for the SES-CD grouped assessments into endoscopic remission, endoscopically mild, moderate, and severe disease. Although statistically significant differences emerged between a majority of the groups in both clinical indices and surrogate laboratory markers, only calprotectin was capable of detecting significant differences between SES-CD remission- and mild- and moderate-activity groups. CRP in turn managed to detect a significant difference between the SES-CD moderate- and severe-activity groups, but on the other hand it failed to distinguish between SES-CD remission and mild activity.

We carried out ROC curve calculations to analyse the power of clinical indices and surrogate markers to detect endoscopic remission. With the SES-CD cut-off value 2 for differentiating endoscopic remission from active disease, the AUC for calprotectin was 0.854. With a calprotectin cut-off value set at the commonly used 100 $\mu\text{g/g}$, sensitivity was 81% and specificity 74%. The level of 94 $\mu\text{g/g}$ was optimized as the best calprotectin cut-off value, with a slightly higher sensitivity of 84% and a specificity of 74% in detecting endoscopic remission. However, the positive predictive value for calprotectin remained low, at 50%, whereas its negative predictive value was 97%. In our search for a normal endoscopic finding, using the SES-CD cut-off value 0, the sensitivity and specificity for calprotectin were quite similar, 82% and 78%, with the optimized calprotectin cut-off value 94 $\mu\text{g/g}$. CRP, CDAI, and HBI were poor at detecting analogous endoscopic remission (**Table 14** and **Figures 5** and **6**).

Table 14. Performance data for clinical indices, faecal calprotectin, CRP, and combined scores for identifying endoscopic remission.

	Cut-off	Sensi- tivity %	Speci- ficity %	AUC	PPV %	NPV %
CDAI , $n=141$	55	71	64	0.730	40	91
HBI , $n=150$	1	80	56	0.729	38	94
Faecal calprotectin ($\mu\text{g/g}$), $n=126$	94	84	74	0.854	50	97
CRP (mg/l), $n=209$	3	50	24	0.643	24	86
HBI + $2 \times \ln[\text{Calprotectin } (\mu\text{g/g})]$, $n=106$	10	85	82	0.900	57	93
Calprotectin ($\mu\text{g/g}$) + $60 \times \text{HBI}$, $n=106$	155	86	82	0.880	60	95
CRP (mg/l) + $3 \times \text{HBI}$, $n=150$	4	80	59	0.766	47	87

AUC: area under the ROC (receiver-operating characteristic) curve; PPV: positive predictive value; NPV: negative predictive value; CDAI: Crohns disease activity index; HBI: Harvey-Bradshaw index; CRP: C-reactive protein; \ln : natural logarithm. All cut-offs are optimized. Data from Study IV.

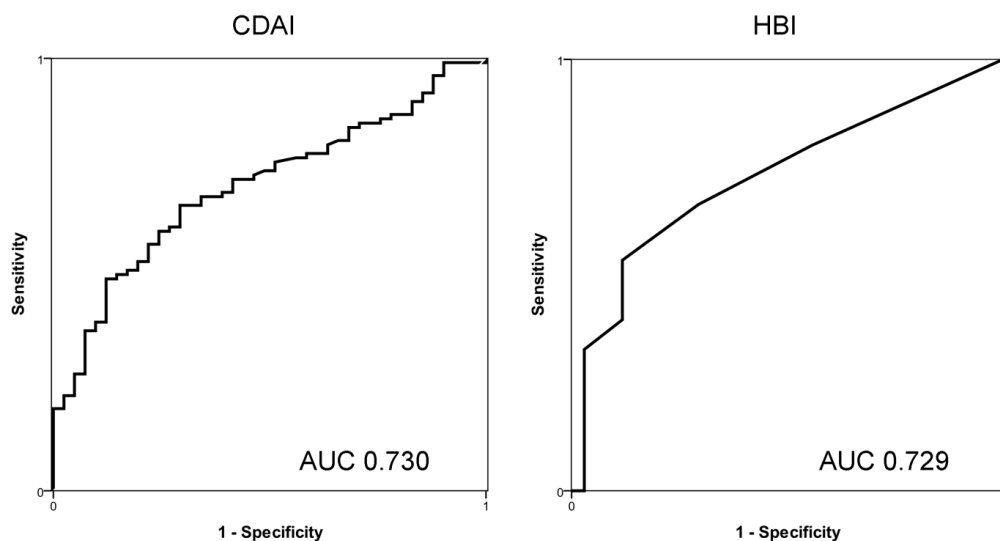


Figure 5. Receiver under the operating characteristic (ROC) curves depicting utility of the CDAI and the HBI as detectors of endoscopic remission, defined as SES-CD 0–2. CDAI: Crohn’s disease activity index; HBI: Harvey–Bradshaw index; SES-CD: Simple endoscopic score for Crohn’s disease; AUC: Area under the ROC curve.

Discrepancies in proportions of patients in endoscopic remission and in clinical remission were also evident in Study II. According to the CDAI, of the 42 patients, 14 (33%) were in clinical remission at the start of anti-TNF, despite their endoscopically active disease; at both follow-ups, a substantially greater proportion were in clinical remission compared with those in endoscopic remission (76% and 73% vs. 24% and 33%). At baseline and at both follow-ups, normal CRP was also clearly more frequent than was endoscopic remission, whereas proportions of patients with normal calprotectin were more similar to proportions with endoscopically determined remission. However, at baseline calprotectin was normal in two patients (73 and 93 $\mu\text{g/g}$) despite their moderate or severe inflammation (SES-CD 11 and 18) according to endoscopy.

3 A non-invasive combination score for detecting endoscopic remission

In Study IV, of 210 disease activity assessments, 126 had available concomitant data on faecal calprotectin and on the SES-CD. Because of the low positive predictive value of a normal calprotectin level ($<100 \mu\text{g/g}$), we analysed further the possibility of improving identification of endoscopic remission by combining existing noninvasive methods. We created ROC curves based on the sums of differently emphasized and combined HBI, CDAI, calprotectin, and CRP and analysed their power to detect endoscopic remission. Scores based on the HBI and calprotectin ($n=106$) proved superior—although without statistical significance—to other combinations and to calprotectin alone. The highest AUC, 0.900, was for the following score: $\text{HBI} + 2 \times \ln[\text{Calprotectin } (\mu\text{g/g})]$. With its sum 10 as the cut-off value for endoscopic remission, its sensitivity was 85%, specificity 82%, positive predictive value 57%, and negative predictive value 93%. We also developed a simplified non-logarithmic score in which the AUC still remained slightly higher than with calprotectin alone (AUC 0.880): $\text{Calprotectin } (\mu\text{g/g}) + 60 \times \text{HBI}$ (**Figure 6**).

In further calculations, defining endoscopic remission as $\text{SES-CD} \leq 3$ only marginally impaired the remission-detecting power of the markers and indices, without changing the superiority of calprotectin and a calprotectin-HBI combination over CRP and the clinical indices. In the search for endoscopies limited to no detectable inflammatory lesions ($\text{SES-CD}=0$), the original combined score $\text{HBI} + 2 \times \ln[\text{Calprotectin } (\mu\text{g/g})]$ still proved superior to that of calprotectin alone; it had an AUC of 0.905, with sensitivity 84% and specificity 83%. Although the clinical indices and CRP, used alone or in combination, proved inferior to calprotectin alone, the score $\text{CRP} + 3 \times \text{HBI}$ seemed to improve identification of endoscopic remission, compared with results of either test separately (**Figure 6**). Performance data for the combined scores are included in **Table 14**.

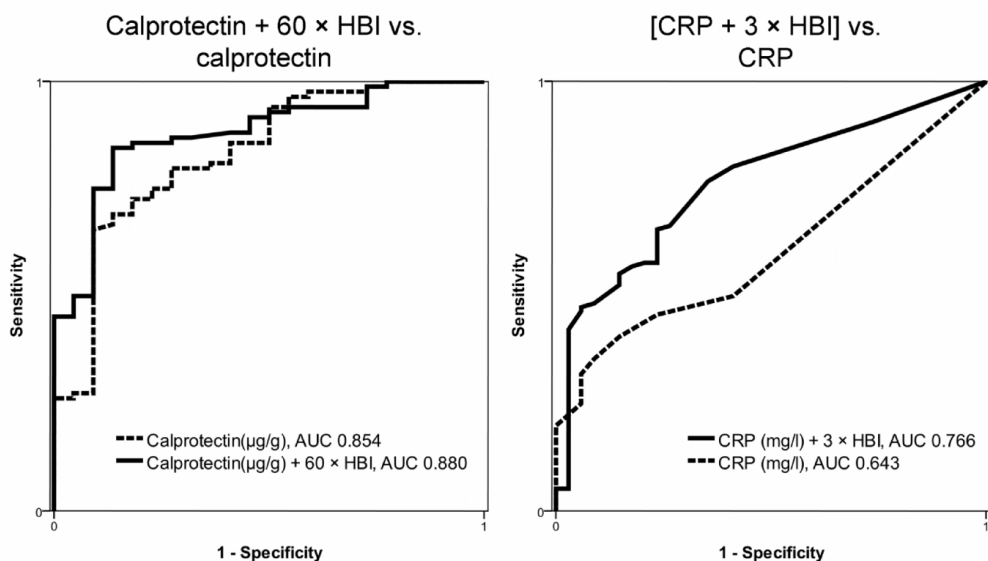


Figure 6. Receiver under the operating characteristic (ROC) curves that demonstrate the power of scores, combining a) faecal calprotectin and the HBI and b) CRP and the HBI, as detectors of endoscopic remission, defined as SES-CD 0–2. Faecal calprotectin and plasma CRP are depicted with dotted lines for comparison. No differences with statistical significance. HBI: Harvey–Bradshaw index; CRP: C-reactive protein; SES-CD: Simple endoscopic score for Crohn’s disease; AUC: Area under the ROC curve.

DISCUSSION

1 Endoscopy as a predictor of long-term endoscopic outcome

The present results, both retrospective and prospective, indicate that endoscopic findings at three months after the start of anti-TNF in patients with luminally active CD are highly predictive for one-year endoscopic response rate. Endoscopic remission achieved at three months is associated with a significantly higher probability of sustained endoscopic remission during maintenance therapy at one year (90% in Study I and 70% in Study II), compared with remission probability if the three-month endoscopic finding demonstrates active mucosal inflammation (33% in Study I and 17% in Study II). The impact of early endoscopic findings on one-year endoscopic response was very similar to that of Hébuterne and coworkers (2013) in the MUSIC study. Use of certolizumab pegol in their study appears the most important difference, though their different endoscopic indices are of marginal relevance due to their strong correlations and their similar definitions for remission.

Early mucosal healing as a predictor of long-term mucosal healing was not, however, as evident in the EXTEND trial. In that prospective, placebo-controlled, double-blind study of CD patients treated with adalimumab, mucosal healing was the primary endpoint. Of 17 patients with mucosal healing at week 12 who received continuous adalimumab, only 8 (47%) had maintained it at week 52 (Rutgeerts *et al.* 2012). This difference may be explained by strict study inclusion criteria comprising a CDAI score of more than 220 points and mucosal ulcers seen at screening endoscopy, and in addition explained by exclusion from primary analysis if patients were switched to open-label adalimumab therapy during follow-up.

Since the beginning of anti-TNF therapy, mucosal healing has become an important predictor of long-term disease outcome in IBD. Mucosal healing during scheduled infliximab therapy reduces need for surgery and hospital treatment significantly (Schnitzler *et al.* 2009). Recently, one study demonstrated that in patients with early-stage CD, complete mucosal healing can lead to significantly higher steroid-free remission rates even for four years after start of therapy (Baert *et al.* 2010). Increasing focus on the concept of therapy quality has also contributed to evolution of treatment goals from endoscopic healing towards deep remission, a combination of clinical and endoscopic remission (Hommes *et al.* 2012, Rutgeerts *et al.* 2012, Travis *et al.* 2012). The EXTEND study sub-analysis demonstrated that all patients who achieved deep remission at week 12 avoided hospitalization during that one-year study. Deep remission was also associated with higher quality of life and lower health-care costs (Colombel *et al.* 2011). Our group, on the other hand, were able to show that endoscopic remission achieved at three months from start of anti-TNF was associated with deep remission at one year. However, as the definition of endoscopically determined mucosal healing is unsettled,

the concept of deep remission must evolve.

Although both the CDEIS and the SES-CD have served in several treatment studies, their numerical cut-off values are insufficiently defined. The rougher CDEIS cut-off between 6 and 7 suggested by Mary and coworkers (2005) has later met criticism. Baseline median CDEIS was 8.8, and scheduled infliximab therapy resulted in a 93% change in the CDEIS at week 54 in an endoscopic substudy of ACCENT 1 (Rutgeerts *et al.* 2004). In another study exploring endoscopic response to infliximab, median CDEIS levels after 10 and 54 weeks of therapy were less than 3 (Geboes *et al.* 2005). The latter study considered CDEIS levels less than 5 as endoscopically mild disease, 5 to 15 as moderate, and above 15 as severe. A CDEIS cut-off between 6 and 7 appears too high, when we consider the definition of mucosal healing as being the complete absence of ulcers, because in only one single bowel segment, one single deep ulcer involving 50% of the surface, plus disease covering 50% without stenosis all results in a CDEIS sum of 4.4 ($[12+5+5]/5$).

Neither the CDEIS nor the SES-CD has been adopted for larger prospective clinical trials. Trials such as ACCENT-1, EXTEND, and SONIC have used the definition “absence of ulcers” as their main endoscopic endpoint (Hanauer *et al.* 2002, Colombel *et al.* 2010, Rutgeerts *et al.* 2012). This definition is tempting and is probably convenient in a study setting, but it is unimaginable that such a strict “black-and-white” endpoint could represent the only relevant treatment goal in clinical practice. The impact of significant endoscopic improvement is also unsettled, although *post-hoc* analysis of the SONIC endoscopic data has suggested that a reduction in ulcer load by half may be as clinically relevant as the complete disappearance of ulcers (Ferrante *et al.* 2011). Studies on validating cut-offs for the SES-CD are limited (Moskovitz *et al.* 2007). Nonetheless, as significant correlations exist between these indices, and as endoscopic remission defined as SES-CD<3 corresponds to the CDEIS<3 definition (Sipponen *et al.* 2010a), use of the more simple SES-CD seems justified both in clinical practice and in the present study.

This study has its limitations. In a purely observational design, our endoscopic and surgical end-points and laboratory and clinical findings present the outcome of a heterogeneous CD patient group during treatment with anti-TNF in real-life clinical practice. Confounding relevant factors are our use of both adalimumab and infliximab and their varying dosages and the small proportion of UC patients in Study III. The differing proportions of patients with endoscopically inactive disease at three months between Study I (45%) and Study II (24%) may at least in part be explained by lack of any validated endoscopic scoring method in Study I. Scoring according to mucosal activity in the most affected bowel segment is likely to produce lower values than values with the SES-CD, whose total score is calculated from sums of variables recorded from up to five bowel segments. Further, no validation of the SES-CD in a postoperative

setting exists, but studies using the SES-CD in similar proportions of patients with a history of surgery are published (Sipponen *et al.* 2010a, b).

Our lack of control groups and our study's observational nature hampered estimation of the real endoscopic impact of anti-TNF therapy, although evidence that anti-TNF maintenance therapy in CD induces mucosal healing significantly more often than does placebo is strong (D'Haens *et al.* 1999b, Rutgeerts *et al.* 2006, 2009). In one small-scale endoscopic study of 22 patients in a placebo-controlled infliximab trial, no endoscopic improvement appeared in the placebo group. Mucosal healing, defined as complete absence of mucosal ulcerations, was documented for almost half at the first follow-up endoscopy. When infliximab was continued with scheduled therapy in patients with an objective initial positive response, mucosal healing was observable in nearly all patients. However, due to the limited number assessed by colonoscopy, no reliable analysis was possible of factors predictive for response (D'Haens *et al.* 1999b). In the ACCENT I endoscopic substudy, with its 99 patients, mucosal healing occurred in 18% with episodic therapy, increasing to 42% in patients on 5 mg/kg dosage, and to 47% on 10 mg/kg scheduled therapy (Rutgeerts *et al.* 2006, 2009). The decision to continue infliximab therapy in the ACCENT I trial was, however, based on clinical responses according to the CDAI (Hanauer *et al.* 2002).

2 Surrogate markers as replacement for endoscopy and in predicting long-term outcome

The emerging need for surrogate markers of mucosal inflammatory activity rises from the numerous drawbacks of endoscopic procedures. Based on our results, we can cautiously claim that in current clinical practice, in active CD, only calprotectin (or another corresponding faecal marker) may be a useful non-invasive surrogate marker for endoscopic assessment of luminal inflammatory activity. It is also useful for distinguishing endoscopically active disease from endoscopic remission. Whereas elevated faecal calprotectin levels are indeed highly predictive of active mucosal inflammation, the utility of low faecal calprotectin levels in predicting inactive disease is still, however, inconclusive (Langhorst *et al.* 2008, Sipponen *et al.* 2008b, Schoepfer *et al.* 2010, D'Haens *et al.* 2012).

Compared to faecal markers, other methods to assess mucosal inflammatory activity in clinical practice appear to be clearly inferior. The CDAI reportedly underestimates endoscopically determined inflammatory activity; therefore, remission determined solely by clinical indices cannot be considered complete (Sipponen *et al.* 2008c). Our study results are similar: differences in endoscopic and established clinical remission rates during anti-TNF therapy in active luminal CD were considerable. If efficacy of therapy means the achievement of endoscopic remission, then our results from anti-TNF

treatment were clearly poorer than if we had defined efficacy as clinical remission. The power of CRP, due to its poor specificity, to recognize endoscopic remission is also far below that of calprotectin. Hence, a normal CDAI or CRP is inefficient in recognizing mucosal healing. However, when comparing inflammation markers, one should also bear in mind that the fistulae and strictures in a considerable proportion of CD patients may have a more pronounced effect on CRP and clinical indices than on calprotectin.

Roseth and coworkers (2004) demonstrated that mucosal healing could be determined by the calprotectin in a simple stool sample. In that study, histological examination of 45 patients with a normal calprotectin showed normal findings for 38; the rest had histologically determined mild inflammation. In a study of 77 CD patients who underwent 106 endoscopic and concurrent stool-marker assessments, Sipponen and coworkers (2008c) demonstrated a significant correlation between faecal calprotectin and the CDEIS ($r=0.729$, $p<0.001$). The CDEIS definition for remission was set at 0 to 2, allowing for only some minor lesions. For use in clinical practice, they suggested calprotectin values <200 $\mu\text{g/g}$ for endoscopically inactive disease (sensitivity 70%, specificity 92%) and values >1000 $\mu\text{g/g}$ for markedly active disease (sensitivity 69%, specificity 93%).

The power of calprotectin to distinguish between mucosal healing and endoscopically active disease is also a topic of Schoepfer and coworkers (2010), who in a study of 122 CD patients tested the hypothesis that calprotectin outmatches other noninvasive tests at making distinctions among patient groups based on severity of endoscopic disease activity. In defining active luminal inflammation as $\text{SES-CD} \geq 4$, sensitivity for calprotectin was 89% and specificity 72% with a calprotectin cut-off >70 $\mu\text{g/g}$, outscoring the CRP sensitivity of 68% and specificity of 58%. In a more recent calprotectin study of IBD and IBS by D'Haens and coworkers (2012), calprotectin levels ≤ 250 $\mu\text{g/g}$ identified endoscopic remission in CD, defined as $\text{CDEIS} \leq 3$, with a sensitivity of 94% and a specificity of 62%. Lobatón and coworkers (2013), who recently analysed stool markers obtained in connection with 115 endoscopies, defined endoscopic remission as a $\text{CDEIS} < 3$. They suggested a calprotectin cut-off as high as 274 $\mu\text{g/g}$. A calprotectin level of >250 $\mu\text{g/g}$ can easily be interpreted as abnormal, whereas the calprotectin endoscopic remission cut-offs reported by D'Inca (2007), Langhorst (2008), and Schoepfer (2010) and coworkers, and the cut-off 94 $\mu\text{g/g}$ in our studies all fall within the usual normal.

Apart from endoscopy, clinically relevant factors predicting long-term mucosal healing are insufficiently clarified. The results of retrospective Study III indicate that a normal calprotectin after induction with anti-TNF is capable of predicting clinical and possibly also endoscopic remission after twelve months of maintenance therapy with anti-TNF in both CD and UC. In comparison to the SES-CD obtained at the same point, by ROC statistics applied to our prospective patient material, calprotectin turned out to be even

more sensitive and specific in predicting twelve-month endoscopic remission. Because of our small number of patients with available data, no statistically significant differences were, however, detectable. Furthermore, our studies reveal the inferiority of the CDAI and of CRP as predictive markers of long-term mucosal healing, compared with results of the SES-CD. The low CRP at week 12 and clinical remission at week 24 as predictive factors for one-year mucosal healing suggested by Kiss and coworkers (2012) await verification. Their very strict definition for mucosal healing (completely normal endoscopic findings), makes any direct comparison with our results quite difficult.

Furthermore, calprotectin appears useful as a predictor of clinical relapse. Calprotectin levels reportedly differ significantly between relapsing and non-relapsing IBD patients (Tibble *et al.* 2000b). Calprotectin may also be a stronger clinical relapse predictor in UC than in CD (Costa *et al.* 2005). On the other hand, a more recent study demonstrates that calprotectin levels $>130 \mu\text{g/g}$ can predict clinical relapse in both UC and colonic CD (D'Inca *et al.* 2008). Comparable findings appear in a study reporting a six-fold increase in relapse within one year in UC cases with baseline calprotectin over $120 \mu\text{g/g}$, and a four-fold increase in CD if the baseline calprotectin level is over $200 \mu\text{g/g}$ (Garcia-Sanchez *et al.* 2010). To the best of our knowledge, no published articles demonstrate the usefulness of faecal calprotectin in estimating CD outcome after induction with anti-TNF. One study, published only as an abstract, reported that normalization of calprotectin correlated significantly with clinical response after anti-TNF induction (Guidi *et al.* 2010). In that study, faecal calprotectin more than $150 \mu\text{g/g}$ after induction therapy indicated increased risk for clinical relapse at one year. This is in line with our results, where a calprotectin cut-off concentration of $139 \mu\text{g/g}$ was capable of distinguishing between a high and a low relapse risk during anti-TNF maintenance therapy. Additionally, our results indicate that one-year outcomes are almost equal between patients with normalized postinduction calprotectin and patients with a considerable postinduction calprotectin decline. The best predictive value for decline in calprotectin in our analysis was 88%. It is therefore reasonable to consider patients with a calprotectin decline of more than 88% as responders, along with those with normal calprotectin levels.

The consequence of our having heterogeneous patients, our differing endoscopic indices and their varying cut-offs for defining endoscopic remission, makes the determination of exact cut-offs for faecal markers in clinical practice a real challenge. In current clinical practice, the use of uniform calprotectin reference values set by the manufacturer may be misleading. Defining a normal or an acceptable calprotectin level for any individual should require knowledge of his or her patient category. The calprotectin cut-off should probably be higher in patients with known inflammatory bowel disease, and lower for merely screening purposes. Cut-offs also possibly need further individual adjustment based on patient history.

3 Identifying endoscopic remission by combining non-invasive markers and clinical indices

A simple combined clinical score, based on calprotectin and the HBI, appeared to be even more specific and sensitive, with better predictive success in identifying endoscopic remission than was calprotectin alone. In clinical practice, this combined score could likely ease the identification of patients in endoscopic remission, as all data required are easily obtained by a simple laboratory test and a brief patient interview. One of the few studies of such noninvasive combined activity indices involved 43 patients with CD; Langhorst and coworkers (2008) here developed a comprehensive activity index for discriminating endoscopically active inflammation from inactive inflammation. This index, comprising the CDAI, CRP, and three stool markers, had a sensitivity of 79% and a specificity of 70%, superior to both CRP and the CDAI, but still inferior to calprotectin alone. Direct comparison of this comprehensive index with our combined score is not feasible, due to several differences: their comprehensive index was based on dichotomous variables, its endoscopic scoring was not based on an endoscopic score, and its cut-off values for both the CDAI (>80) and the laboratory markers (calprotectin >48 $\mu\text{g/ml}$, CRP $>7\text{mg/l}$) differed.

The combined score, however, reveals a paradox. Several studies have demonstrated that in IBD patients with bowel symptoms, a normal calprotectin level is helpful in distinguishing IBD from IBS and ruling out active disease (Tibble *et al.* 2000a, D'Inca *et al.* 2007, Kaiser *et al.* 2007, Langhorst *et al.* 2008). This finding is already well implemented in clinical practice. Yet the present study identified CD patients with apparently normal calprotectin levels but with active bowel inflammation revealed by endoscopy. In several of these patients, the combined score indicated active disease because of the rise in HBI score derived from subjective symptoms. This phenomenon may be explained by natural fluctuation in daily calprotectin levels and the fact that such a “normal” calprotectin based on laboratory reference values may, in a subgroup of CD patients, still be abnormal. This is a reminder of the importance of observing CD as a whole, not overlooking subjective symptoms in patients with objectively, but only non-invasively, suggested quiescent disease.

The new combined score was developed in patients originally receiving anti-TNF. Because many disease-activity assessments occurred during no anti-TNF exposure at all, its use should be unquestionable in patients with luminal CD independent of type of therapy. With the combined score's failure to reach statistical superiority to that of calprotectin alone, recognition of endoscopically active disease in those patients with normal calprotectin and mild subjective symptoms remains a challenge. The value of the combined score, as demonstrated here, must be confirmed in prospective series; further research into other combined scores is essential. A valid combined score would be an important, simple, and cost-effective tool in CD treatment, to direct endoscopy resources to those patients with active disease.

CONCLUSION

Today, when medical treatment of CD increasingly is based on efficient but expensive highly immunosuppressive anti-TNF, achievement of an objective treatment response and identification of reliable predictive markers would provide an optimal cost-benefit ratio. Although endoscopy remains the treatment of choice for objective assessment of mucosal inflammation, the need exists to replace endoscopy with noninvasive surrogate markers. Based on the present work, objective disease-activity assessment of mucosal healing, performed as early as three months after start of anti-TNF, appears to offer the best prognostic evaluation of treatment in luminal CD. Additionally, both a normal calprotectin and a considerable calprotectin drop from baseline are promising as predictive markers for long-term remission. By combining calprotectin, its elevation as a reliable marker for endoscopically active disease, and a clinical index such as the HBI, detection of endoscopic remission may improve. Adapted for clinical practice, these data would suggest routine assessment of faecal calprotectin at baseline and at the three-month follow-up after initiation of anti-TNF, with low-threshold referral to endoscopy in suspected nonresponders within a year after initiation of therapy.

The question of objective treatment response is still open, however, due to limited data on validated cut-off values for remission and response from endoscopic indices and faecal calprotectin. Even were mucosal healing regarded as the primary goal in treatment of CD, differentiating among other disease activity levels is also necessary in assessment of treatment response, particularly in those patients unable to achieve endoscopic remission.

FINNISH AND SWEDISH SUMMARIES

Yhteenveto

Tausta

Crohnin tauti on krooninen, useimmiten paksusuolella ja ohutsuolen loppuosassa esiintyvä suolitulehdus. Sen tyypillisiä oireita ovat vatsakivut, laihtuminen, veriset ulosteet ja ripuli, jotka pahimmillaan johtavat pysyvään työkyvyttömyyteen. Taudin vaikeampia ilmentymiä voidaan hoitaa tuumorinekroositekijä α :n vasta-aineilla (anti-TNF), jotka monessa tapauksessa ovat riskialttiita ja joista vain osa potilaista hyötyy.

Tutkimusten mukaan paksusuolen tähytyksellä osoitettava limakalvon paraneminen ennustaa vahvasti suotuisaa pitkäaikaishoitoavastetta. Hoitovasteen seuranta voi kuitenkin olla haastavaa, koska suolen limakalvon tulehdusaktiiviteetti ei välttämättä korreloi potilaan subjektiivisten oireiden kanssa.

Koska tähytys on aikaavievä, kallis ja monelle potilaalle kivulias tutkimus, on kehitetty kajoamattomia korvaavia menetelmiä tautiaktiiviteetin arvioimiseksi. Korvaavien merkkiaineiden, kuten ulosteen kalprotektiinin, kyky tunnistaa tähytyksellä osoitettava limakalvoparaneminen, on riittämättömästi selvitelty. Limakalvoparanemista ennustavia tekijöitä anti-TNF-hoidon aikana on myös vajavaisesti tutkittu.

Potilaat ja menetelmät

Väitöskirjatyo koostui neljästä osatyöstä (I–IV). Pitkäaikaiseen limakalvoparanemiseen liittyvien tekijöiden löytämiseksi analysoitiin sekä takautuvasti (I: 71 potilasta, III: 60 potilasta) että prospektiivisesti (II: 42 potilasta) kerättyä tietoa anti-TNF-hoidetuilta Crohnin tautia sairastavilta potilailta. Lisäksi selviteltiin mahdollisuuksia korvata paksusuolen tähytys kajoamattomilla merkkiaineilla ja kliinisillä oireindekseillä (IV: tiedot yhteensä 210 tähytystutkimuksesta).

Tulokset

Limakalvoparaneminen kolme kuukautta hoidon aloituksesta oli vahva ennustetekijä sen jatkumiselle vielä vuoden jälkeen. Myös normaali kalprotektiini noin kolmen kuukauden hoidon jälkeen ennusti suotuisaa vastetta vuoden jälkeen. Kalprotektiini oli paras yksittäinen merkkiaine tunnistamaan samanaikainen limakalvoparaneminen. Uusi, tutkimusryhmän kehittämä, kliinisestä oireindeksistä ja kalprotektiinista koostuva yhdistelmäindeksi osoittautui vielä kalprotektiinia herkemmäksi ja tarkemmaksi limakalvoparanemisen tunnistamisessa.

Päätelmät

Paksusuolen tähytys tai kalprotektiinin määrittäminen kolme kuukautta hoidon aloituksesta optimoi anti-TNF-hoitoa Crohnin taudissa. Kliinistä oireindeksiä ja ulosteen kalprotektiinia yhdistelevän uuden indeksin avulla voidaan paremmin ohjata rajalliset tähytysresurssit sitä eniten tarvitseville potilaille.

Sammanfattning

Bakgrund

Crohns sjukdom är en kronisk tarminflammation som oftast drabbar tjocktarmen och slutet av tunntarmen. Vanliga symtom är buksmärta, viktninskning, blodig avföring och diarré, vilket i värsta fall leder till arbetsoförmåga. Sjukdomen kan behandlas med antikroppar mot tumörnekrosfaktor α (anti-TNF), en i många fall riskabel behandling som endast en del patienter drar nytta av.

Forskning visar att en i koloskopi påvisbar läkning av tarmslemhinnan är starkt kopplad till en gynnsam prognos. Uppföljningen av behandlingsresponsen är dock utmanande, eftersom tarmslemhinnans inflammatoriska aktivitet inte nödvändigtvis korrelerar med patientens subjektiva symtom.

Eftersom koloskopi är en tidskrävande, dyr och ibland smärtsam undersökning, har man utvecklat ersättande noninvasiva metoder för att uppskatta sjukdomsaktiviteten. Surrogatmarkörer, bland annat det fekala kalprotektinets, förmåga att upptäcka endoskopiskt påvisbar slemhinneläkning är dock otillräckligt utredd. Likaså är prognostiska faktorer för långvarig slemhinneläkning under anti-TNF-behandling bristfälligt klarlagda.

Patienter och metoder

Avhandlingsprojektet bestod av fyra delarbeten (I–IV). För att identifiera faktorer kopplade till långvarig slemhinneläkning analyserades både retrospektivt (I: 71 patienter, III: 60 patienter) och prospektivt (II: 42 patienter) insamlade uppföljningsdata från patienter med anti-TNF-behandlad Crohns sjukdom. Utöver detta utreddes möjligheter att ersätta koloskopi med surrogatmarkörer och kliniska symtomindex (IV: data från sammanlagt 210 endoskopiundersökningar).

Resultat

Slemhinneläkning tre månader efter att behandlingen börjat var en stark prognostisk markör för fortsatt slemhinneläkning vid ett år. Ett normalt fekalt kalprotektin efter cirka tre månaders behandling förutspådde också en gynnsam ettårsprognos. Fekalt kalprotektin var den bästa enskilda surrogatmarkören för att upptäcka slemhinneläkning vid samma tidpunkt. Ett nytt, av forskningsgruppen utvecklat kombinerat aktivitetsindex som består av ett kliniskt aktivitetsindex och kalprotektin, uppvisade en ännu högre sensitivitet och specificitet för att upptäcka slemhinneläkning.

Slutsatser

Koloskopi eller kalprotektinmätning tre månader efter att behandlingen börjat optimerar anti-TNF-behandlingen vid Crohns sjukdom. Ett noninvasivt kombinationsindex bestående av ett symtomindex och kalprotektin kan bidra till att de begränsade koloskopiresurserna koncentreras till de patienter som verkligen behöver genomgå koloskopi.

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